Comparative study of the clinical manifestations and pathophysiology of leptospirosis and scrub typhus

Thesis

Comparative study of the clinical manifestations and pathophysiology of leptospirosis and scrub typhus

Wirongrong Chierakul

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To all leptospirosis and scrub typhus victims

Wirongrong Chierakul, MD

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Glossary of terms

AFI	Acute febrile illness
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
APACHE II score	Acute physiology and chronic health evaluation II score
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under curve
AUFI	Acute undifferentiated febrile illness
BUN	Blood urea nitrogen
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase-MB (mass)
CSF	Cerebrospinal fluid
CT ratio	Cardiothoracic ratio
CXR	Chest x-ray or chest radiograph
D-dimer	cross-linked fibrin degradation products
DIC	Disseminated intravascular coagulation
F1+2	Fragment 1,2
FCT	Fever clearance time
GI	Gastrointestinal
IFA	Indirect immunofluorescent antibody test
IQR	Interquartile range
LDH	Lactate dehydrogenase
LEST score	Leptospirosis-scrub typhus score
MAP	Mean arterial pressure
MAT	Microscopic agglutination test
MCAT	Microcapsule agglutination test
OR	Odd ratio
PT	Prothrombin time
PUO	Pyrexia of unknown origin
RR	Respiratory rate
SOFA score	Sepsis-related organ failure assessment score
sv.	Serovars
TAT	Thrombin-antithrombin III complexes

VPW	**Vascular pedicle width**
vs	**Versus**

Publication arising from this thesis

Chierakul W, Tientadakul P, Suputtamongkol Y, Wuthiekanun V, Phimda K, Limpaiboon R, Opartkiattikul N, White NJ, Peacock SJ, and Day NP. Activation of the coagulation cascade in patients with leptospirosis. *Clin Infect Dis* Jan **2008**; 46(2):254-60.

Abstract

The study aims were to determine the prevalence of leptospirosis and scrub typhus in northeast Thailand, and to compare and contrast their clinical features and outcomes. Leptospirosis and scrub typhus accounted for 45% of acute febrile illness in 1249 prospectively studied patients presenting to Udon Thani hospital, northeast Thailand between October 2000 and December 2002, nearly 20% of who had leptospirosis and scrub typhus coinfection.

A total of 311 patients had leptospirosis, 97 of who were culture-positive for *Leptospira* spp. The culture-positive group had fewer complications, including aseptic meningitis, jaundice, renal impairment, thrombocytopenia and pulmonary haemorrhage, compared with the culture-negative group. A total of 304 patients were diagnosed as having scrub typhus, one-fourth of who had putative reinfection based on the presence of an IgG response without detectable IgM. Patients with primary scrub typhus infection were significantly younger and presented to hospital later than patients with reinfection. Primary infection was associated with jaundice, liver impairment and gastrointestinal bleeding, but fewer patients had shock and confusion compared with patients with reinfection.

Patients with leptospirosis had significantly more hepatic and renal impairment, thrombocytopenia and bleeding diathesis than patients with scrub typhus. Despite this, the mortality rate was comparable at around 3% for each infection, and the major cause of death for both diseases was pulmonary haemorrhage. Patients with concurrent leptospirosis and scrub typhus had more severe clinical features (shock, jaundice, renal failure, thrombocytopenia and bleeding diathesis) than patients with one of these diseases alone, but mortality was comparable.

A leptospirosis-scrub typhus (LEST) score based on clinical features and routine laboratory tests was developed to predict the diagnosis of leptospirosis or scrub typhus. The score specificity approached 90% for both diseases. Further validation of the LEST score is required to determine its accuracy in routine clinical practice and in other geographic areas.

Chapter I: Introduction

1.1 General background

Leptospirosis is a worldwide zoonosis caused by pathogenic spirochetes of the genus *Leptospira*. A variety of wild and domestic animals excrete the organism in their urine, and human infection occurs through direct contact with infected animals or through exposure to fresh water or soil contaminated by infected animal urine.[117] Leptospirosis has recently been recognised as an 'emerging infectious disease' in many countries including Thailand. The annual number of reported cases has been increasing in many hospitals and recent outbreaks of the disease have been reported in Thailand since 1997.[5]

The clinical manifestations of leptospirosis vary greatly, ranging from a mild flu-like illness, often self-limiting, to an acute life-threatening condition. Only patients with symptomatic forms of the disease come to medical attention, so the number of patients affected by this disease may be underestimated.

Scrub typhus is also a zoonotic disease, caused by *Orientia tsutsugamushi*, a Gram-negative obligate intracellular coccobacillus transmitted to man and other vertebrates via the bite of larval stage trombiculid mites (or Chiggers). Human disease is endemic in the Asia-Pacific region from Korea to Papua New Guinea and Queensland, Australia, and from Japan to India and Afghanistan.[240] An estimated 1 billion individuals are exposed and 1 million cases occur each year. Mite larvae survive in damp soil or detritus in scrub vegetation associated with disturbed habitats including the margins of agricultural lands and edges of forests, riverbanks and abandoned rice fields, and disease is most often associated with occupations which expose individuals to these environments.

Leptospirosis and scrub typhus, taken together, account for almost 60% of all acute febrile illness in northeastern Thailand. Major presenting features of both diseases are similar and include fever, headache and myalgia.[261] A proportion of patients with scrub typhus develop a pathognomonic papule at the bite site that ulcerates to form a black crust or eschar associated with drainage lympadenopathy.[37] In both diseases patients may develop conjunctival suffusion. Disease severity and manifestations within any given population vary widely from asymptomatic to fatal. However, scrub typhus is a mild disease in most cases. Patients with severe disease may develop renal failure, jaundice, abnormal liver function tests, hypotension and pulmonary haemorrhage, but complicated or fatal disease is rare in the post-antibiotic era.[37,326] This contrasts with leptospirosis which results in severe disease and multi-organ involvement in 10%, of whom 25% die. Accurate diagnosis of scrub typhus relies on serological testing using an indirect immunofluorescence test. This is usually performed on paired sera taken about two weeks apart. By its nature, this provides a late, retrospective diagnosis. The ability to make a clear clinical distinction between the two infections would make an important contribution towards appropriate patient management, and would alert the clinician as to the likely complications and outcome.

1.2 Acute febrile illness

In the rural tropics acute undifferentiated febrile illness is a common clinical syndrome among patients presenting at many hospitals, and this is especially true in the northeast of Thailand. The actual pathogenic causes of acute febrile illness (AFI) are generally overlooked. Many patients are treated empirically to cover the potential diagnoses based on the clinical presentations. Knowledge of local epidemiology patterns of disease is critical to inform treatment decisions made by clinicians and health care providers. In many areas, the prevalence and incidence of infectious diseases are unknown, with estimates based on local diagnostic capabilities, individual anecdotal reports and the specific interests of the local personnel. Rapid diagnostic tools for tropical infectious diseases are usually unavailable for most diseases, unreliable, need careful interpretation, and relatively expensive. The new molecular diagnostic tools require an investment in training, infrastructure, and technology that is unrealistic in many rural areas.

1.2.1 Aetiologies of acute febrile illness in Asia

Asia is the largest continent, and consists of various temporal zones including tropical areas and temperate regions. The epidemiology of the causative agents of infectious diseases in each country is very variable, depending very much on the climatic and geographical conditions.

In Nepal for example, bloodstream infection caused by *Salmonella typhi* is a major cause of fever especially in summer. Leptospirosis and scrub typhus occur in 2-5% and 3%, respectively.[188] The two most important diseases causing AFI in Egypt have been reported as typhoid fever and brucellosis, the latter occurring infrequently in the East part of Asia.[121]

In 1957, leptospirosis accounted for 35% of military personnel with AFI in Malaya, and scrub typhus and malaria were also common in the same series accounting for 11% and 9% respectively.[184] In the same report, but in civilian patients, leptospirosis, scrub typhus, malaria and dengue infections were found in 13%, 3%, 7%, and 24%, respectively.[184] A subsequent study on febrile patients in rural Malaya also showed that scrub typhus was the most frequent diagnosis (19.3%) especially among oil palm labourers, followed by typhoid and paratyphoid (7.4%). Leptospirosis accounted for 6.8%, and malaria was found in 6.2%.[37]

1.2.2 Aetiologies of acute febrile illness in Thailand

In Thailand, acute pyrexia of unknown origin (acute PUO) is one of the communicable diseases required to be reported to the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. It has been suggested that the terminology 'acute PUO' in the reporting system should be changed due to the fact that it does not conform to the definition of classical PUO.[166] These patients present with abrupt fever of less than 2-weeks duration and non-specific symptoms and illness disappear in almost all cases within 1 to 2 weeks after any treatment or no treatment, and neither definite diagnostic laboratory tests were confirmed or performed. The term acute undifferentiated febrile illness (AUFI) was proposed for use instead of acute PUO.

AUFI was reported to affect approximately 200,000-350,000 patients each year (**Figure 1.1**). This unduly large reported incidence reflects the poor quality of national disease surveillance data regarding the occurrence of febrile illnesses, mainly because of the deficiency of diagnostic tools and a lack of effort to obtain a definite diagnosis. It is often judged to be a poor use of resource to use costly diagnostic tools on a self-limiting or easy-to-manage patient in a developing country which although not poor is not yet a well-equipped setting.

Aetiological studies of acute febrile illness from various parts of the country are very important and helpful to inform management and antibiotic policies.

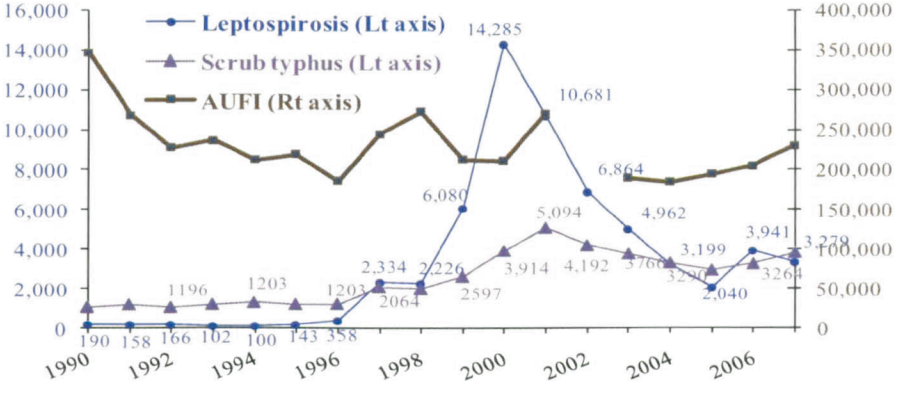

Figure 1.1 Number of acute undifferentiated febrile illness (AUFI) [on right axis], leptospirosis and scrub typhus [on left axis] in Thailand between 1990 and 2007.

(Source: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, http://epid.moph.go.th)

On the Thai-Myanmar border, which is an endemic area for malaria infection, malaria was unsurprisingly the major cause (one-quarter) of acute fever. Leptospirosis was found in 17.5% of patients and all rickettsioses were found to account for nearly 6%. The major rickettsiosis found in this area was not scrub typhus, but spotted fever group (SFG) rickettsioses, which are rarely reported in Thailand.[87]

In the years 1991-1993, the surveillance data from 10 community-based hospitals representing all parts of Thailand showed that the three most common causes of AFI were scrub typhus (7.5%), influenza (6.0%) and dengue fever (5.7%), with leptospirosis occurring in only 1.1%.[167] These figures contrast with the report conducted nearly a decade later, in July 2001-June 2002, in which leptospirosis became a major cause (36.9%) of adult AFI in 5 hospitals in Thailand (3 hospitals in the northeast, and one each in the south and the centre), and scrub typhus (16%) was the second most common disease found.[261] The great differences between these two reports can be

explained by several factors. Firstly, there were major differences between the studies in the populations and the geographical areas studied, the age group of patients, and very importantly the diagnostic measures used. In the former study, leptospirosis was diagnosed using a MAT test in which only one representative serogroup, Bataviae, was studied, whereas in the latter study, the MAT tests were performed using a much larger panel of 23 representative serogroups, and blood culture for *Leptospira* was also performed. Secondly, there was an outbreak of leptospirosis in Thailand starting from the year 1997 to 2002 as shown in **Figure 1.1**, so the proportion of patients with leptospirosis was dramatically increased.[5]

1.3 Leptospirosis

1.3.1 History of leptospirosis

Leptospirosis is a zoonotic disease caused by spirochetes in the Genus *Leptospira*. The disease is believed to have existed since ancient times, as a clinical description appeared in several languages at various time periods with different names all over the world. Most of the names or the descriptions of the disease mentioned illnesses, mostly fever and jaundice, linked with the rainy season, plantation, flooding, water exposure, environmental or occupational exposure. It was described in several parts of China, Japan, and much later in Europe, America, Africa, and Australia.[92]The clinical descriptions by Hippocrates, Galen and Avicenna, the famous Fathers of Medicine, also mentioned this disease entity. The disease was clearly described for the first time in 1812, as 'fièvre jaune' among Napoleon's troops in 1800, during the siege of Cairo.[161] Several epidemics also recorded in other parts of the world including Asia Minor, Syria, Egypt, and France. This was before the germ theory of infection was developed, so it is difficult to judge whether these were true leptospirosis cases or other disease entities which cause similar manifestations, such as plague, malaria, yellow fever, viral hepatitis, scrub typhus, typhoid, etc.

The disease was really first recognised by Professor Adolf Weil in the year 1886.[314] He described 4 cases of a febrile illness and severe nervous symptoms, hepato-splenomegaly, jaundice and signs of renal disease all of which recovered rapidly. By the time he published his report, the clinical, pathological and epidemiological sciences had progressed sufficiently to accept the recognition of new diseases. The disease often occurred among troops, which were present in a large number throughout Europe at that time. Though he could not demonstrate the pathogenesis and the causative agent, this disease entity was later on named 'Weil's disease'. Since much of the medical literature

in Europe was in the German language, name Weil's disease was adopted rapidly and became well known in other parts of the world.[253]

The causative agent of leptospirosis was first described in a patient who died of a disease thought erroneously to be yellow fever.[253] Stimson used the Levaditi technique to stain spirochetes in the brain, liver, heart and kidney tissue of yellow fever patients, and found hook-ended, spirochaete-like organisms in the cells and lumens of renal tubules, but not in the blood vessels, glomeruli or interstitial tissues, nor in other organ tissues. He suggested the name of the organism as *Spirochaeta interrogans* due to the form of the organism which resembled a 'question mark'. Later on Inada and colleagues isolated the organism from the blood and transmitted the infection to guinea-pigs. He named the organism *Spirochaeta icterohaemorrhagiae*, and described the mode of infection, distribution of the organism in the tissues, excretion of the spirochaete, filterability and morphological characters.[119] The same group also demonstrated that rats played a role as carriers of the disease and this led to a better understanding the epidemiological principles of transmission by animal vectors and the search for other animal sources of the disease.[117] It was recognised later on that leptospirosis accounted for several disease entities recorded in various parts of the world, and was a veterinary problem in food animal production.[233]

1.3.2 The genus *Leptospira*

The extensive studies in the early phase of *Leptospira* discovery were conducted by Noguchi who grew the organism from the liver of a patient who died of yellow fever in Ecuador. He named the organism *Spirochaeta icteroides* and classified the organism into the same group (genus *Leptospira*) as *Spirochaeta icterohaemorrhagiae*, the causative agent of Weil's disease.[196-198] It was proved later on that the two organisms were identical and not the cause of the mosquito-transmitted 'yellow fever'.[99,232,276]

Within the genus *Leptospira* (family *Leptospiraceae* of the order *Spirochaetales* under the phylum *Spirochaetes*) there are many pathogenic and non-pathogenic species. The most common known pathogenic *Leptospira* in the group are *L. interrogans*, *L. fainei*, *L. kirschneri*, and *L. borgpetersenii*, and the most common non-pathogenic one is *L. biflexa*. A recent classification based on molecular studies has led to division of *Leptospira* into a number of genomospecies as shown in **Figure 1.2**, which is not fully congruent with the older serological classification.[169] However the classification based on immunological reactions is still used in serological diagnostic tests such as the MAT, and still has relevance in diagnosis, epidemiology and when considering prevention strategies such as vaccination.

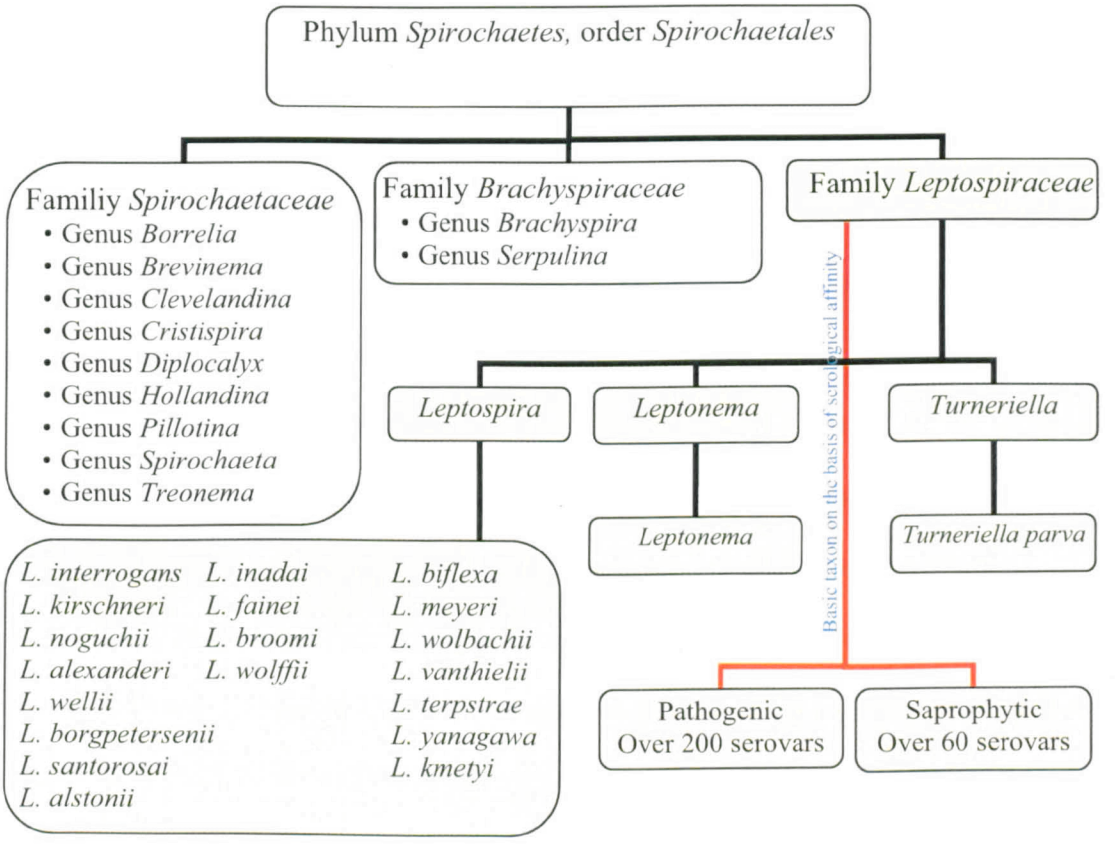

Figure 1.2 Classification of *Leptospira*

1.3.3 Epidemiology of leptospirosis

1.3.3.1 The geographical distribution of leptospirosis

After the discovery of the causative agent and the detailed clinical description of the disease, numerous bacteriological and serological reports of the disease followed from most countries and regions of the world. Leptospirosis has been recognised by the World Health Organization (WHO) as one of the most widely spread zoonotic diseases.[3] In Asia, apart from China and Japan in ancient times, the disease was first described in the Netherlands East Indies in 1923,[19] followed by the Malay States in 1927,[96] the Andaman Islands in 1931,[270] French Indo-China in 1934,[20] and Indonesia in 1939.[296]

The incidence of human infection is higher in the tropics than in temperate regions, but disease occurs in both industrialised and developing countries. Lack of awareness and inadequate diagnostic tools together with the occurrence of symptomless or subclinical infection in the natural course of the disease may distort the real figures for the true incidence of the disease.

There are two main types of study concerning the extent of the disease, aetiological studies of patients with AFI (hospital-based) and population-based serological surveillance (community-based), which require different interpretations.

The geographical distribution of leptospirosis as described in a recent published review is shown in **Figure 1.3**.[209] The annual incidence of leptospirosis derived from official data reported by individual countries from 1996 onwards, ranked by incidence, is shown in **Table 1.1**.[209]

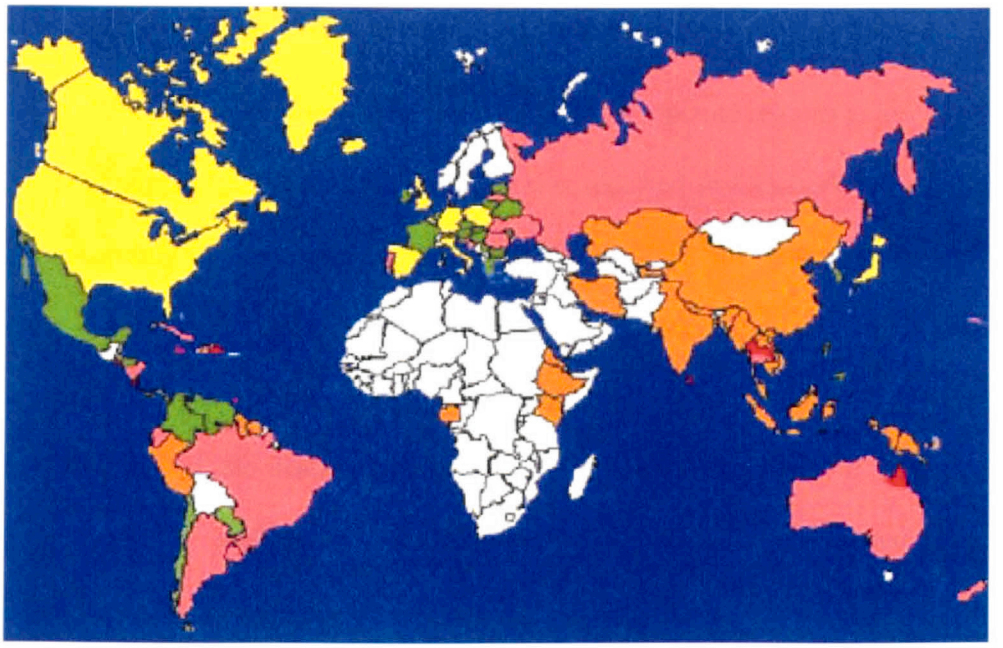

Figure 1.3 Global annual incidence of human leptospirosis.

Colours reflect incidence, in declining order: red (>2 per 100,000 population), pink (1-2 per 100,000 population), green (0.2-1 per 100,000 population), and yellow (<0.2 per 100,000 population). The orange reflects areas with probable, but not estimated high incidence. White reflects absence of data.[209]

Table 1.1 Annual incidence of leptospirosis worldwide

Countries with the highest incidence			Countries for which no data are available, probably endemic	Other countries	
Rank	Country	Annual incidence per million population		Country	Annual incidence per million population
1	Seychelles	432.1	India	Belarus	3.4
2	Trinidad and Tobago	120.4	Malaysia	Bulgaria	3.7
3	Barbados	100.3	Bangladesh	Chile	1.6
4	Jamaica	78	Vietnam	Colombia	1.6
5	Costa Rica	67.2	Laos	Czech Republic	1.8
6	Sri Lanka	54	Nepal	France	3.9
7	Thailand	48.9	Cambodia	Germany	0.7
8	El Salvador	35.8	Indonesia	Greece	3
9	New Zealand	26	Myanmar	Honduras	3.1
10	Uruguay	25	China	Hungary	3.1
11	Cuba	24.7	Iran	Ireland	2.2
12	Nicaragua	23.3	Suriname	Italy	0.7
13	Croatia	17.3	Haiti	Lithuania	2.2
14	Russia	17.2	Peru	Mexico	1
15	Ukraine	15.3		Netherlands	1.9
16	Dominican Republic	13.8		Panama	1.3
17	Brazil	12.8		Paraguay	1.9
18	Ecuador	11.6		Serbia and Montenegro	1.5
19	Argentina	9.5		Singapore	2
20	Romania	9.4		South Korea	2.8
21	Australia	8.9		Spain	0.3
22	Portugal	6.8		UK	0.6
23	Denmark	6		USA	0.1
24	Latvia	5.6		Venezuela	3.8
25	Slovenia	5.4			
26	Philippines	4.8			
27	Slovakia	4.4			
28	Taiwan	4.1			

1.3.3.1.1 Leptospirosis in temperate regions

Overall the occurrence of leptospirosis in temperate regions is low. For example an incidence of 0.09/100,000 population was reported in Denmark for 1970-1996, but the mortality was relatively high at up to 7%; all cases were due to the Icterohaemorrhagiae serovar.[109] Human cases appear not to occur in Canada, and leptospirosis was removed from the notifiable diseases list in the USA in 1995, despite the existence of the disease in Hawaii with an annual incidence of 1.29 per 100,000 population[134,187] and sporadic case reports associated with recreational activities (for example a large outbreak among athletes participating in triathlons in Illinois and Wisconsin in 1998).[187] Awareness of the disease and disease surveillance in USA is obviously needed since parts of USA are also in the temperate zone.

In Europe, the incidence of the disease has not changed significantly over the past decade. Most of the cases and the annual incidence depend on specific circumstances occurring in the area, such as flooding or recreational activities including water sports associated with submersion in natural water reservoirs. The overall annual incidence is much lower when compared to the incidence in tropical regions.

1.3.3.1.2 Leptospirosis in tropical regions

Most parts of the tropics are endemic for leptospirosis, including the Caribbean, Central and South Americas, Southeast Asia and Oceania. However underreporting or absence of reports is a major problem in evaluating the accurate incidence of leptospirosis in many countries.

Many studies and experts in leptospirosis are from India; hence the disease is viewed there as a real major public health problem. In fact, there is no official incidence data from most parts of India, and the same is true for Bangladesh, Pakistan and Nepal.

A neighbourhood country, Sri Lanka, carries a very high annual incidence reported by the Ministry of Public Health.[209]

The Seychelles (43.2) in the Indian Ocean, Trinidad and Tobago (12.0) and Barbados (10.0) in the Caribbean had the three highest annual incidences of leptospirosis per 100,000 population reported anywhere in the world.[209] This conforms to previous studies done in Caribbean schoolchildren carried out at various locations and times for a variety of reasons (testing for poliovirus protective immunoglobulin, parasitological screening, or yellow fever surveys). The *Leptospira* seropositivity ranged from 6.0% in the Bahamas to 36.6% in Barbados.[90] There were 125 patients (incidence 101/100,000 population) with confirmed-leptospirosis in a population-based study in the Seychelles during 1995-1996.[327]

1.3.3.1.3 Leptospirosis in Southeast Asia

There is considerable evidence that leptospirosis is endemic in certain areas of Southeast Asia despite the lack of official reports. The overall *Leptospira* seropositivity was 18.8% among 1,400 subjects in a large cross-sectional study in Southern Vietnam performed in early October, the flooding season in Mekong Delta. The most prevalent serogroups were Bataviae, Panama, Icterohaemorrhagiae and Australis.[291] In a recent study in children from Vietnam, 10.4% had seroconverted to *Leptospira* IgG, with a rate of seroconversion of 0.99%/year.[271] The percentage of *Leptospira* seropositivity in a community-based study in a rural area along the Mekong River in Lao PDR was nearly 24%. The most prevalent serogroups were Autumnalis, Hebdomadis, Icterohaemorrhagiae and Panama.[135]

In Cambodia the evidence base on leptospirosis epidemiology is extremely limited. Based on a study of 121 suspected cases of leptospirosis, in which only 4 patients could be confirmed by MAT (2 Javanica and 2 Australis), the annual incidence

was estimated at around 7.65/100,000 population.[234] On the other hand an earlier evaluation of patients with AFI among displaced Khmer people residing in temporary settlements on the Thai side of Thai-Cambodian border over a one month period showed no patient with leptospirosis.[80]

1.3.3.2 Seasonal incidence of leptospirosis

Leptospira are capable of surviving in the free state in natural water sources for a period of time. Their survival in this free-living state very much depends on the environmental conditions. The optimum conditions for leptospires are moisture, warmth (about 25°C), and around neutral pH values for soil and surface water. These conditions are often found in tropical areas all year round, and during the summer and autumn in temperate zones.[287]

Seasonal variation in the incidence of human leptospirosis has been reported in several countries. Spatial analysis of 488 cases presenting in Brazil between 1997 and 2002 shows that clustering of cases were 3.7 time more likely to occur following heavy rainfall.[268] In Italy, where most of the cases occur in the Northern regions of the country, the peak of incidence is during the summer.[59,60]

In animals, the risk of seroconversion of cows against *L. grippotyphosa* was significantly higher during spring in Spain, while the risk of seroconversion against *L. bratislava* did not differ significantly between the seasons.[105] Leptospirosis in dogs in the USA and Canada in 1983-1998 also demonstrated a seasonal distribution, with cases occurring in late summer to fall with peaks following rainfall.[301] This was confirmed by the same author in the USA during 1997-2002; seroconversion of dogs against *L. kirschneri* serovar Grippotyphosa was common in summer, and hospital cases of leptospirosis with the same serovar was common in the fall.[302]

1.3.3.3 Mode of transmission

Several outbreaks or sporadic occurrences restricted to risk exposures associated with specific occupational groups were reported from various parts of the tropics. The high risk groups are farmers, abattoir workers, livestock farmers, miners, sewer workers, eco-tourists, water sportsmen or activities involving immersion in water.

That leptospires adhere to the tubular epithelial cell border of the renal tubules in the animal carrier is very crucial for the epidemiology of leptospirosis, since this facilitates excretion of leptospires into the environment with animal urine. Human are almost always incidental and dead-end hosts who acquire the disease through contact with water, soil or mud that is contaminated with infected urine, or direct contact with infected animals or animal excreta.

1.3.3.3.1 Skin, conjunctiva and mucosal membrane

The most common portal of entry is through skin or conjunctiva. Leptospires can enter intact skin, but more easily enter scratched or abraded skin or skin sodden from prolonged immersion in water. Entry through the conjunctiva usually occurs by splashing or aerosolisation of contaminated excreta or water, and entry through mucosal membranes in the respiratory tract could occur via inhalation of contaminated water or aerosols.[159]

1.3.3.3.2 Oral route and water-borne transmission

Outbreaks of leptospirosis following drinking of contaminated water from wells, fountains or ponds have been reported in Portugal (126 cases), Greece (31 cases), Russia (62 cases) and Italy (35 cases).[168] One fountain-related outbreak in Italy in July 1984 caused three deaths (including two unconfirmed cases), and 32 non-fatal confirmed cases of leptospirosis. The causative *Leptospira* was in the Australis

serogroup, and the source of *Leptospira* contamination was probably a hedgehog trapped in a reservoir of water not in use but still connected to the water system of the fountain.[41]

A recent outbreak in a nurse hostel in Chennai, South India, which affected 35% of the residents over a 5-week period was also proved to be cause by contamination of an underground storage water tank with *Leptospira* of the Icterohaemorrhagiae serovar.[227]

1.3.3.3.3 Intrauterine infection and congenital leptospirosis

In animals intrauterine infection causing abortion or stillbirth is very common, but occurs infrequently in human. The possible outcomes are abortion (especially in the first trimester), stillbirth, dead fetus in utero, or progression to normal maturity and birth. The birth of a healthy baby from a pregnant woman with leptospirosis in Israel was reported in 1993, and two more cases were reported earlier.[235] Abortion occurs more commonly, whereas congenital infection is rare. Leptospirosis should not be considered as an indication for termination of pregnancy. Awareness of the possibility of infection, especially in pregnant women in epizootic areas or pregnant women who have a history of travel to endemic areas, is of utmost important for early detection and prompt treatment and for the safety of the foetus.

1.3.3.3.4 Sexual transmission

Human to human spread of leptospirosis is very rare, though humans can transiently shed leptospires into urine after infection. Two consecutive cases of leptospirosis occurred in an airman canoeing in a rat infested area in Australia and his wife who just watched him from the bank and did not handle the wet canoe or clothing.[107] This raised the possibility of post-coital infection, since the serology of both patients was positive for the same serovar, Icterohaemorrhagiae.

1.3.3.3.5 Breast milk transmission

Milk from cattle is a major source of transmission from animals to animals, or from animals to human during the milking process, but again human to human transmission via this route is very rare. One breast fed baby contracted leptospirosis from his mother who was recovering from a Hardjo leptospirosis infection.[30]

1.3.3.3.6 Laboratory-acquired infection

Three cases of leptospirosis acquired from laboratory exposure have been reported from the National Animal Disease Centre, mainly by direct contact with infected animals.[256] Working with leptospires in culture and with laboratory animals are both risks and standard laboratory safety precautions should be applied. Baseline titres of *Leptospira* antibody should be established on employment, and the reports of 'influenza' illness should be monitored for the possibility of the development of leptospirosis. Prophylactic immunisation is not generally available except in China.[37] Recent report from India emphasises the possibility of accidental acquisition of infection during laboratory work, especially when dealing with high concentrations of organisms in culture.[255]

1.3.4 Leptospirosis in Thailand

1.3.4.1 History of leptospirosis in Thailand

The first report of leptospirosis in Thailand was published in 1943,[331] and was followed by several series of case reports. Following flooding in Bangkok between 1948 and 1950 there were 52 reported cases of leptospirosis, five of whom died.[257]

In 1964 fifty-four patients with leptospirosis were admitted to a Medical University Hospital in central Bangkok, 37 of who were confirmed by haemoculture onto Fletcher's medium, lysis agglutination, or animal inoculation. The majority of the

causative *Leptospira* were of the Bataviae serogroup (35/54[64.8%]), and 2 cases (3.7%) were of the Icterohaemorrhagiae serogroup. The mortality was 7.4%.[47]

1.3.4.2 Current leptospirosis situation in Thailand

A sero-epidemiological survey in people residing in North and Northeast Thailand in the year 1983 revealed that 0.27% were seropositive for *Leptospira* antibody, whereas the seropositivity in rodents in the areas was much higher; 11.5% of *Bandicota indica* and 7.4% of *Rattus rattus*.[40]

The number of leptospirosis cases in Thailand ranged from 100-400 cases/year during the 40 years prior to 1996. In 1997, the number of cases suddenly increased to over 2,000 cases/year, reached 6,000 cases/year in 1999 and peaking in the year 2000, in which more than 14,000 cases (40 times increased from 1996). The overall mortality rate was 2.5%. The number of cases declined in the years 2001 to 2005, and then stabilised at around 2,000-4,000 cases/year (10 times higher than the incidence prior to the outbreak) (**Figure 1.1**). The age distribution of patients is shown in **Figure 1.4**. Eighty-five percents of the patients were in the northeastern part of the country (**Figure 1.5**).[5] In the year 2000, there was also an outbreak of leptospirosis in Had Yai City, Songkhla, Southern Thailand, after a big flood in the town. Leptospirosis accounted for 27.2% of acute febrile illnesses occurring in children following the flood.[219]

Isolates from a prospective study conducted between 2000-2005 in Udon Thani and from human cases occurring in other provinces during the same period demonstrated that a single *L. interrogans* serovar (Autumnalis) was responsible for this sustained outbreak. Using a novel multilocus sequence typing (MLST) scheme these isolates were shown to be clonal (most of them having the sequence type (ST) 34).[273]

The important reservoirs for leptospirosis identified so far in Thailand are rodents and shrews, 5.6% of which were positive for *Leptospira* antibodies.[154]

1.3.4.3 Seasonal incidence of leptospirosis in Thailand

The leptospirosis incidence data in Thailand clearly demonstrates seasonal variation related to the rainy season, which is between July and October (**Figure 1.6**). A study of samples from patients with suspected leptospirosis from 9 provinces representing all parts of Thailand was able to confirm the diagnosis in only 20% of cases, and most of these occurred between July and October (**Figure 1.7**).[320]

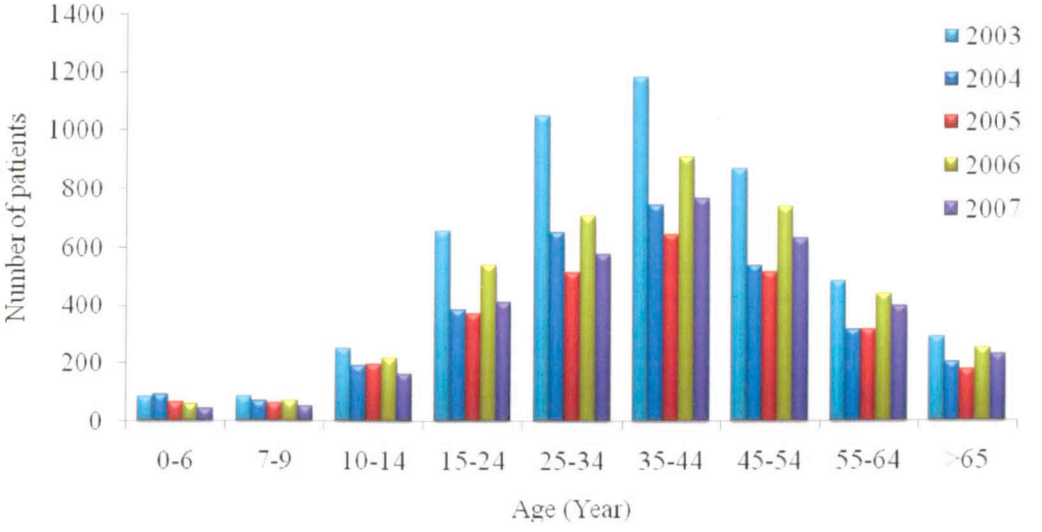

Figure 1.4 Age distribution of patients with leptospirosis in Thailand, 2003-2007

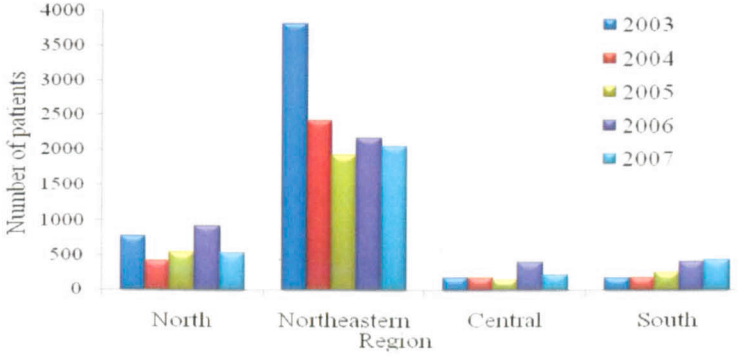

Figure 1.5 Distribution of patients with leptospirosis according to regions in Thailand, 2003-2007

(Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, http://epid.moph.go.th)

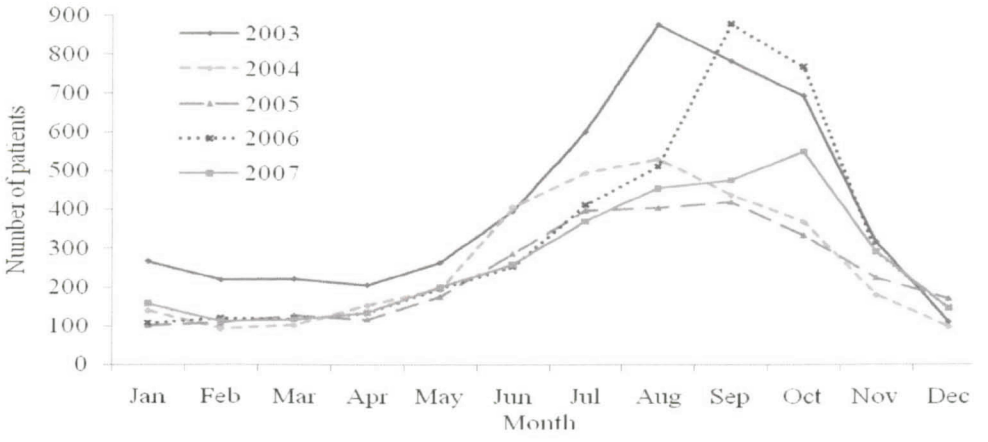

Figure 1.6 Number of patients with leptospirosis in Thailand per month from 2003-2007

(Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, http://epid.moph.go.th)

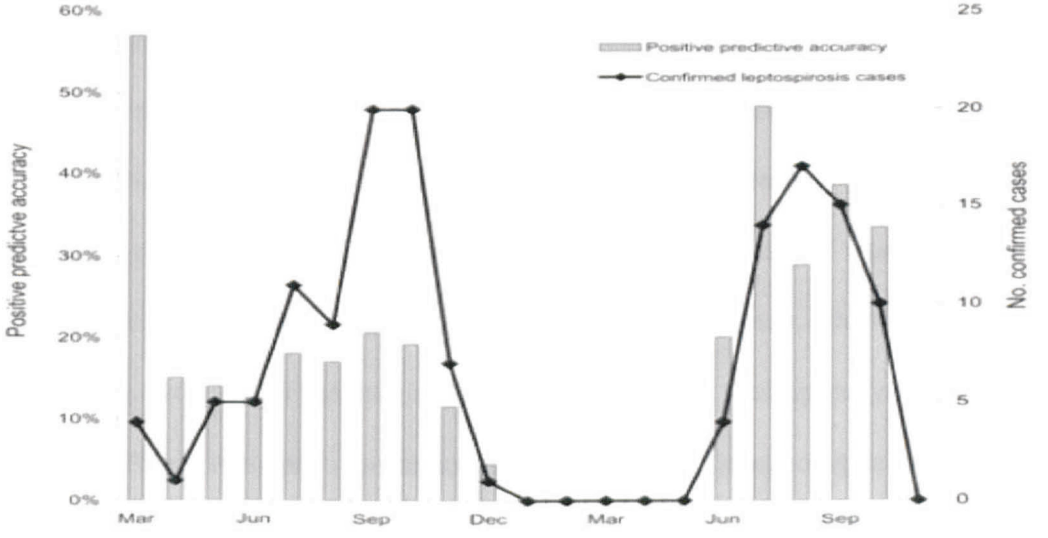

Figure 1.7 Cases of laboratory-confirmed leptospirosis and positive predictive accuracy of clinical diagnosis by month, Thailand, March 2003-2004[320]

1.3.5 Pathogenesis

The incubation period can be as short as two days and as long as a month. After *Leptospira* enter the body via skin or mucosal membranes their ability to survive depends on their ability to escape the innate and acquired immune responses. Leptospires do not cause an acute inflammatory response in the tissues in which they reside, so individual leptospires or significant clumps can exist and multiply without

eliciting inflammation in the human body. They appear not to activate recognition factors by interaction of their cell walls with tissue mediators of inflammation, therefore the cellular infiltration in the lesions is probably a response to the trauma and tissue injury. Leptospires spread almost immediately from the site of entry, via the lymphatic system to the bloodstream, and from there circulate to all tissues. They grow exponentially in the bloodstream and tissues, with an approximate doubling time of eight hours. The main factor affecting the length of the incubation period is the size of inoculation.[92]

The central pathologic change characteristic of all forms of leptospirosis is damage to the walls of small blood vessels, leading to the leakage and extravasation of cells and to haemorrhages. It is postulated that the pathogenesis of leptospirosis is due to the direct toxicity of the cell components of leptospires themselves.[12] Specific putative virulence factors of leptospires include: the phospholipases, which can cause haemolysis; glycolipoprotein (GLP), which is cytotoxic to various cell types and may play an important role for renal failure in leptospirosis; peptidoglycan, which can induce tumour necrosis factor (TNF)-alpha production in monocytes; and lipopolysaccharides (LPS).[92] The LPS of leptospires has structural, chemical and immunological properties similar to those of other Gram negative bacteria, but is much less lethal in animal experiments. It is also highly immunogenic. LPS may play an important role in the pathogenesis of thrombocytopenia, platelet aggregation and adhesion to vascular endothelium and fibrinolysis, leading to severe fatal pulmonary haemorrhages.[190]

1.3.6 Clinical manifestations

Leptospirosis is considered to be a systemic infectious disease with a broad range of clinical symptoms. These clinical manifestations arise from the effects of a generalised vasculitis, the central pathogenesis of the disease. Patients may have very

mild insignificant symptoms with a self-limiting course. There is no accurate estimate of the extent of this group of patients, but it is believed to be the great majority of leptospirosis infections. A small proportion of patients present with an acute febrile illness and other constitutional symptoms, and even fewer patients present with severe complications.

Patients who are severely ill enough to seek medical attention almost always present with fever. Other constitutional symptoms accompanying fever may be present, but none of them are specific to the disease. At the time of the onset of symptoms it may not be possible to tell clinically whether the disease will be mild or severe. A review of clinical symptoms and signs of leptospirosis in reports from various parts of the world is shown in **Table 1.2**.

1.3.6.1 Asymptomatic or subclinical infection

A substantial proportion of people infected with leptospirosis may have very mild, unnoticed or subclinical disease and do not seek medical attention. Evidence for subclinical infection comes from serological surveys performed in many endemic areas. In the Seychelles, 37% and 9% of screened people had past and recent infections respectively, though none of them reported any recognisable symptoms of leptospirosis.[33]

1.3.6.2 Biphasic pattern

In 1967, Turner published a description of the clinical presentation of leptospirosis as having a biphasic pattern; an acute or septicaemic phase lasting about a week, followed by an immune phase characterised by the presence of antibody and leptospiuria.[287]

The changing pattern of clinical presentations of leptospirosis has cast doubt on the accuracy of this biphasic description of leptospirosis, but the description has been

referenced in every textbook and articles onwards. The utility of this view may be of importance only when considering laboratory testing.

1.3.6.2.1 Acute or septicaemic phase

Leptospiraemia usually lasts no longer than a week. Patients almost always present with a febrile illness of sudden onset. Other symptoms accompanying the fever during this phase are chills, headache, severe myalgia, conjunctival suffusion, anorexia, nausea, vomiting, and prostration. Fever then subsides for few days before the start of symptoms from the immune phase. For patients suspected of leptospirosis with a clear risk history who present to hospital within the first week of illness, blood, or CSF culture inoculated into special media for leptospires may be very useful. The antibody is theoretically undetectable, so rapid screening tests based on antibody detection may not be very helpful.[27,168,287]

1.3.6.2.2 Immune phase

This phase starts in the second week of illness. Characteristic of this phase is the presence of antibody against *Leptospira* and the presence of leptospires in urine. Most of the complications of leptospirosis are associated with localisation of leptospires within the tissues during this phase. Fever may recur and rise to 40°C, accompanied by more severe headaches, rigors and chills and worsening prostration. This second phase is usually not obviously separate, merging with the first phase into a single continuous illness. Renal and hepatic complications, bleeding diatheses and aseptic meningitis may occur at this stage.[27,92,168,287]

1.3.6.3 Classification of the severity of clinical symptoms

The clinical manifestations of leptospirosis have also been classified, based on the severity of clinical symptoms, into a number of forms.

1.3.6.3.1 Anicteric leptospirosis

This is the mildest presentation, though complications can occur during the immune phase. Aseptic meningitis may be found in less than a quarter of patients. Moreover, pulmonary haemorrhage may complicate the course of disease and contribute to the mortality in this relative non-severe form of leptospirosis.

1.3.6.3.2 Icteric leptospirosis

Icteric leptospirosis is a severe form of leptospirosis characterised by jaundice. The clinical course of the icteric form usually progresses rapidly. Other complications may coincide with jaundice including renal failure, bleeding diathesis (especially pulmonary haemorrhage), and cardiac involvement. The incidence of icteric form varies according to reports ranging from 16% to more than 90% (though some studies which may concentrate only the severe form of leptospirosis) (**Table 1.2**).

1.3.6.3.3 Weil's disease

This is the most severe form of infection, characterised by the presence of jaundice, renal failure and haemorrhage. The classic Weil's disease as originally described differs somewhat, consisting of jaundice, renal failure, hepatosplenomegaly and neurological symptoms.[314] This severe form occurs in around 5% to 10% of patients with leptospirosis who present to the hospital, and has a mortality ranging from 5% to 40%.

1.3.6.4 Symptoms and signs

1.3.6.4.1 Constitutional symptoms

The headache symptoms in leptospirosis are almost always severe and of sudden onset. Retro-orbital pain and photophobia may occur resembling that occurring in dengue infection. Rigors or chills may accompany the fever. Anorexia, nausea and

vomiting also occur, as they do in most febrile illnesses. None of these symptoms are specific for leptospirosis. The differential diagnosis at this stage of the disease process is inevitably broad and local epidemiological data can be very helpful.

1.3.6.4.2 Myalgia, myositis, arthritis

Muscle pains and concomitant tenderness may be excruciating. In addition to generalised pains in the neck, abdomen, and limbs, there may be particularly severe muscle pains, especially in the calf muscles, thigh and back. The symptoms are found in between 40%-100% of cases, depend on the series reported. In Korea and Barbados, myalgia occurred in 40% and 49%, respectively, while 97% and 100% were found in Puerto Rico and China, respectively.[6,58,85,212] Life threatening rhabdomyolysis may occur infrequently: 5% in 58 cases with renal failure in Moldova;[65] two cases reported from the Netherlands;[290] and one case reported each from Hawaii,[64] Vanautu,[199] and Brazil.[68] The condition was reported in a surprisingly high 52% of 46 cases in a series from Israel.[28] The criteria used for diagnosing rhabdomyolysis in this report were questionable.

The severe muscle pain in leptospirosis results from a characteristic degeneration of striated muscle, causing extreme tenderness and spasm.[11,237] Additional findings include haemorrhages, loss of cross-striations, myocyte swelling, and vacuolation surrounded by inflammatory cells. *Leptospira* antigen was demonstrated in the muscle lesions.

Although half of patients may have arthralgia, arthritis occurs rarely. Reactive arthritis involving spine, shoulders, and knees was reported in a man 3 weeks after discovery from leptospirosis pneumonitis and lung abscess.[316]

Table 1.2 Symptoms and signs of patients with leptospirosis in large case series

Symptoms and signs	Chung[57]	Charoonruangrit[47]	Alexander[6]	Wang[299]	Berman[24]	Park[212]	Edwards[85]	Yersin[327]	Ko[152]	Katz[133]	Mansour-Ghanaei[180]	Vanasco[292]
Number of patients	75	54	208	168	150	93	88	75	193	353	74	182
Study period	1955	1962	1963	1965	1973	Sep-Dec 1987	1990	Apr 1995-Mar 1996	Mar-Nov 1996	1974-1998	Jun-Sep 1999	Jan 1999-Dec 2005
Place	China	Bangkok, Thailand	Puerto Rico	China	Vietnam	Chonbuk, Korea	Barbados	Seychelles	Salvador, Brazil	Hawaii, USA	Guilan, Iran	Argentina
Mean Age (±SD) or median (range)	na	na	na	na	na	na	na	36±15.6	36±15.2	33 (1-78)	47.3±12.4	32±16.2
Sex: male	na	47(87%)	na	na	na	na	na	63 (84%)	153 (79%)	325 (92%)	52 (70%)	155 (87%)
History of fever	na	89%	na	na	na	97%	na	96%	94%	99%	99%	94%
Headache	89%	89%	91%	90%	98%	70%	76%	80%	75%	89%	93%	87%
Myalgia	100%	91%	97%	64%	79%	88%	49%	63%	94%	91%	77%	78%
Arthralgia	51%	na	na	36%	na	na	na	31%	na	59%	na	na
Conjunctival suffusion	97%	94%	99%	57%	42%	58%	54%	38%	29%	28%	41%	55%
Anorexia/dehydration	92%	67%	na	46%	na	80%	85%/37%	na	na	82%	na	na
Nausea/vomiting	56%/51%	54%/48%	75%/69%	29%/18%	41%/33%	46%/32%	37%/50%	na/40%	na	77%/73%	74%/70%	na
Abdominal pain	31%	26%	na	26%	28%	40%	43%	41%	na	51%	58%	na
Diarrhoea	30%	na	27%	20%	29%	36%	14%	11%	na	53%	12%	na
Hepatomegaly	83%	67%	69%	28%	15%	17%	27%	na	na	16%	na	na
Lymphadenopathy	19%	na	24%	49%	21%	na	21%	na	na	na	na	na
Rash	0	4%	6%	na	7%	na	2%	na	na	8%	na	na
Jaundice	72%	52%	49%	§	§	16%	95%	27%	93%	39%	69%	50%
Respiratory symptoms	na	na	na	na	na	na	na	na	na	17%	na	22%
Cough	55%	37%	24%	57%	20%	45%	32%	39%	na	na	31%	na
Haemoptysis	37%	na	9%	51%	na	40%	na	13%	20%	na	5%	na
Nuchal rigidity	na	11%	na	na	na	1%	na	na	5%	27%	26%	13%
Photophobia	na	na	na	na	na	na	na	na	na	na	20%	na
Oliguria	na	na	na	na	na	na	na	na	33%	26%	na	na

§Among anicteric leptospirosis

na: not available

Table 1.3 Laboratory findings and outcome of patients with leptospirosis in large case series

Laboratory findings	Charoonruangrit[47]	Park[212]	Yersin[327]	Ko[152]	Katz[134]	Mansour-Ghanaei[180]
Place	Bangkok, Thailand	Chonbuk, Korea	Seychelles	Salvador, Brazil	Hawaii, USA	Guilan, Iran
Anaemia	na	37%	na	na	32%	78.4%
Thrombocytopenia	na	na	28%	na	58%	87.3%
Proteinuria	59.3%	43%	na	na	54%	79.0%
elevated BUN	na	na	na	na	49%	na
elevated creatinine	na	15%	28%	na	54%	na
elevated bilirubin	na	21%	48%	na	70%	na
elevated AST	na	50%	na	na	na	na
elevated ALT	na	39%	na	na	73%	na
Mortality	7.4%	na	8%	5.2%	1.4%	0

*Among anicteric leptospirosis

na: not available

BUN: Blood urea nitrogen

AST: Aspartate aminotransferase

ALT: Alanine aminotransferase

1.3.6.4.3 Renal involvement

Though renal failure is an important complication of leptospirosis, symptoms may be inapparent or even absent in mild cases. Oliguria may occur in the leptospiraemic phase, with the presence of red cells, albumin, leucocyte or granular casts in urine. In the early phase of disease, dehydration may be the major factor causing the derangement of renal function, reflected by a high level of BUN which can be greater than 70 mmol/L. Renal insufficiency can occur within the first 3-4 days of illness, followed by a rapid rise of BUN and creatinine.

Renal pathology consists of renal swelling and interstitial nephritis. In severe case, tubular necrosis and medullary tubular cell desquamation can occur. Ischemia of renal cortex, dilatation of medullary vessels and medullary haemorrhages were apparent. The glomeruli are usually spared, though exudate and inflammatory cell infiltrations have been reported in some cases.[12]

1.3.6.4.4 Liver involvement

Jaundice is an important manifestation of Weil's disease, the most severe form of leptospirosis, and actually occurs in only 5-10% of all patients. Jaundice in leptospirosis is associated with the cholestasis of sepsis rather than hepatocellular damage. It usually appears on days 4-6, up to 9 days after onset. An enlarged liver may be encountered in many patients. Jaundice in leptospirosis is not associated with pruritus.[228]

The pathology in liver consists of increasing of hepatocytes and binucleated hepatocytes, spotty necrosis, surrounded by mononuclear cells, without swelling or ballooning of the hepatocyte. The Kupffer cells and sinusoidal lining cells may also be involved with hypertrophic cytoplasm bulging into the lumen of sinusoids. Stasis of bile is shown by bile droplets in the cytoplasm of hepatocytes and bile thrombi in the

canaliculi, evidenced mostly in the centrolobular areas of the lobules.[26] Complete liver cell necrosis is not prominent in human leptospirosis. Bile capillaries show microvilli disappearance or distortion on electronmicroscopic study, suggestive of a nonspecific finding seen both in the extra and intrahepatic forms of cholestasis.[76] Increase in circulating haemoglobin and bilirubin from the breakdown of erythrocytes from the haemorrhagic area and from haemolysis contribute to the degree of jaundice.

1.3.6.4.5 Cardiovascular involvement

Cardiac involvement in leptospirosis may occur more commonly than previously thought. An incidence of around 10% was reported in 1951,[247] but in a pathologic study in fatal patients 70% of patients had histological evidence of cardiac involvement,[72] and electrocardiographic (ECG) abnormalities were found in 86% of 239 ECGs from patients with acute leptospirosis.[224]

Pathologic studies show that cardiac involvement may range from small areas of focal inflammation to diffuse inflammation with interstitial oedema. There are lymphocytic and plasma cells infiltrations; leukocytes are usually seen only in small numbers, are most commonly detected in the atria, atrioventricular region, and interventricular septum, accompanied by petechiae or larger area of haemorrhages.[72]

1.3.6.4.5.1 Conduction abnormalities and cardiac arrhythmias

Various pattern of conduction abnormalities have been found in patients with leptospirosis. Intraventricular conduction delay, nonspecific T-wave changes, first–degree artioventricular blockage, and ST-T wave changes suggestive of acute pericarditis are all reported as common ECG abnormalities.[309] Marked transient ST segment elevation in anterior chest leads has also been reported in severe icteric leptospirosis.[226] These abnormalities often return to normal after proper antibiotic treatment.

Acute atrial fibrillation was the most common major cardiac arrhythmia found in leptospirosis (14%).[225] Despite the common conduction system abnormalities occurring in patients with leptospirosis, left ventricular function of the patients appeared to be normal on echocardiographic and Doppler examination.[225] Other cardiac arrhythmias can occur including atrial flutter, premature ventricular contractions, and ventricular tachycardia. Cardiac arrest from arrhythmias can occur and may be the cause of death, but the majority of these abnormalities resolve after treatment.

1.3.6.4.5.2 Myocarditis

In an autopsy series of patients who died from leptospirosis the cardiac pathology showed lymphocytic and plasma cells infiltrates consistent with myocarditis in 50% of cases.[72]

1.3.6.4.5.3 Pericarditis

The true prevalence and aetiology of pericarditis in patients with leptospirosis are still unknown, since autopsies failed to find significant pericardial inflammation. Frequent physical findings for this condition are the presence of a pericardial friction rub and ST-T changes characteristic of pericarditis, such as ST-T wave elevation. This was frequently found in conjunction with the presence of severe renal failure, so whether the condition was directly due to leptospirosis or due to uraemic pericarditis was unclear.

1.3.6.4.5.4 Congestive heart failure

This appears to be an infrequent complication of leptospirosis. On chest radiographic studies cardiomegaly occurred in less than 10% of patients.[164,247] A case report of patient from Hawaii with jaundice, thrombocytopenia and hypotension had sinus tachycardia with intraventricular conduction delay and diffuse, nonspecific T-

wave change, and chest radiographs showed mild cardiomegaly and diffuse bilateral interstitial infiltrates. Echocardiographic study of the patient showed a mildly left ventricle dilatation with overall depression of systolic function and no segmental wall motion abnormalities, reflecting true congestive heart failure rather than pericarditis as the cause of cardiomegaly.[78]

1.3.6.4.5.5 Aortitis and arteritis

Aortitis was found in nearly 60%, and coronary arteritis affecting the main branches of the coronary arteries was observed in 70% of patients who died from leptospirosis in one study reported since 1987. Consistent with pathology in other areas, lymphocytic and plasma cells infiltrates predominated. Despite substantial inflammation, actual thrombosis of the coronary arteries was rare.[72]

1.3.6.4.6 Neurological involvement

1.3.6.4.6.1 Meningitis

In leptospirosis true meningitic neck stiffness may be difficult to differentiate from muscle pain associated with myalgia. Aseptic meningitis is seen in up to 25% of patients, and usually appears in the immune phase or the second week of illness. Abnormalities in CSF include increased opening pressure, raised protein, normal glucose, and lymphatic pleocytosis.

CSF abnormalities may occur more commonly than the presence of symptoms and signs of meningism. Cargill and Beeson performed CSF examination in 14 adult patients with fever, jaundice and serology positive for leptospirosis during the years 1943-1946, and observed that all except one of them had abnormal findings.[42]

A case of meningitis occurred in a sewer worker five days after submerging the lower half of his body for two hours in a sewer. The *Leptospira* serovar was Andamana,

identified by cross-agglutination-lysis test and cross-absorption test. The Andamana serovars is found in *L. biflexa*, which is believed to contain saprophyte, non-pathogenic *Leptospira*.[150]

A cluster of three cases of leptospirosis presented 18 days after an accident on a boat rafting tour with headache, meningism and facial paralysis. Two patients had antibodies against *Leptospira interrogans* serovar Bataviae.[252] Though many serovars can cause meningitis, some serovars may be specifically neurotropic.

1.3.6.4.6.2 Other uncommon neurological manifestations

Patients with leptospirosis can present with uncommon manifestations involving the neurological system. These include polyneuropathies, Guillian-Barré syndrome, mononeuritis multiplex, cranial nerve palsies, cerebrovascular disease, and peripheral nerve palsies.[66,75]

1.3.6.4.7 Unusual presentations

1.3.6.4.7.1 Erythema nodosum

An 8-year-old girl with a history of dog bite developed high fever and painful, raised erythematous areas on both shins mimicking erythema nososum; serologically she was diagnosed as having a *Leptospira* serovar Canicola infection.[77] A 12 year old boy diagnosed as having leptospirosis presented with high fever, malaise, aseptic meningitis, severe bradycardia and erythema nodosum was reported a year later.[39]

1.3.6.4.7.2 Generalised muscle weakness

A rare case report was reported from India of progressive generalised muscle weakness leading to quadriplegia and respiratory failure a few days before other clinical symptoms and signs of leptospirosis.[157] The weakness was the consequence of hypokalaemia and resolved after this was corrected. Hypokalaemia in leptospirosis can

occur secondary to abnormal sodium and chloride transportation in the proximal tubule, but not in the distal tubule. The sodium load in the unaffected distal tubule results in the loss of potassium in the exchange for sodium. Hypokalaemia may therefore occur in patients with or without azotaemia.[177]

1.3.6.4.7.3 Acute pancreatitis and acute cholecystitis

Acute pancreatitis has been reported rarely in leptospirosis, though the serum amylase may be raised in up to 65% of patients with severe leptospirosis, especially in those with jaundice and renal failure.[85] Concomitant pancreatitis and cholecystitis are not uncommonly reported.[138,186] Pancreatitis may be misdiagnosed as cholecystitis,[213] and severe muscle pain in leptospirosis may also lead to the misdiagnosis of an acute surgical abdomen leading to unnecessary surgery. Acute pancreatitis in a patient with fatal anicteric leptospirosis has also been reported.[251]

1.3.6.4.7.4 Leptospirosis and pregnancy

Leptospirosis in pregnancy can result in abortion, intrauterine foetal death,[61] and congenital leptospirosis.[235] Postpartum haemorrhage and foetal death caused by maternal leptospirosis has also been reported.[279]

1.3.7 Pulmonary leptospirosis

The true incidence of pulmonary involvement is unclear but may range from 20%-70%. It is usually mild and often overlooked, but may be very serious and fatal.[200] The severity of pulmonary involvement is unrelated to the presence of jaundice.[164,212,280]

1.3.7.1 Respiratory symptoms and signs

Patients may present with symptoms ranging from cough, dyspnoea, and haemoptysis, to adult respiratory distress syndrome.

Respiratory symptoms occurred in up to 85% of military personnel in Malaya, with cough as the most common abnormality.[184] Among patients who had cough, bloody sputum was recognised in 25%. In Korea, blood tinged sputum or haemoptysis occurred in 40% of patients in an outbreak after severe flooding in 1987.[212] The majority of patients' sera were reactive to *Leptospira* antigens of the Icterohaemorrhagiae serogroups.[211]

1.3.7.2 Chest radiographs

The description of three radiological findings in leptospirosis was noted among 64% of patients with leptospirosis in Korea: small nodular infiltrates, large confluent areas of consolidation, and diffuse, ill-defined, ground-glass appearance. Abnormalities usually occur bilaterally and are non-lobar, with a marked tendency toward peripheral predominance.[118]

Park et al. suggested the classification of 3 forms of chest radiographs abnormalities in leptospirosis: severe (markedly increased, patchy and localised confluent, predominant acinar nodules); moderate (moderately increased, diffusely patched or some nodular, predominant alveolar nodules or military densities, widely disseminated); and mild (slightly increased, reticulonodular or some mottled, predominant interstitial lung marking). The cut-off for each category was subjective and depended on the reader. In 93 cases in an outbreak following flooding, 40 (43%) had abnormal chest radiographs. The majority of them showed bilateral lesions, with right-sided predominance. Pleural effusion and cardiomegaly were seen in 30% and 33%, respectively. All but 2 cases were improved 3-9 days after antibiotic treatment, without scarring or sequelae.[212]

In Malaya, chest radiographs were abnormal in only 11 out of 42 patients, varying from slight increases in vascularity to diffuse infiltrates with small localised areas of increased density.[184]

1.3.7.3 Severe pulmonary haemorrhage

Pulmonary haemorrhage has been increasingly recognised throughout the world over the past decade as a fatal manifestation of leptospirosis. In several outbreaks the mortality related to pulmonary haemorrhage, for examples Korea,[212] Brazil,[67,194] and Nicaragua.[2] The outbreak in Nicaragua in 1995 after heavy flooding was characterised by massive pulmonary haemorrhage, including fatal sudden haemoptysis, without jaundice or renal manifestation, leading to diagnostic confusion with epidemic haemorrhagic fever of viral origin (such as Hantaan virus and dengue infections).[2,280]

Pathological changes observed in the lungs of patients who died from pulmonary haemorrhage caused by leptospirosis consisted of fibrin aggregates suggestive of adult respiratory distress syndromes in nearly 60% of cases, capillary lesions characterised by swollen endothelial cells with an increase in pinocytotic vesicles and giant dense bodies in the cytoplasm, and haemorrhagic pneumopathy with septal capillary lesions. No necrosis, rupture, nor exposed subendothelial collagen was observed outside haemorrhagic areas (characteristic of DIC).[194]

1.3.8 Laboratory findings in leptospirosis

1.3.8.1 Complete blood count

Anaemia is a prominent finding in leptospirosis, especially in severe cases, and is probably due to haemorrhage and haemolysis. Haemolytic anaemia associated with leptospirosis in animals is due to haemolysins with phospholipase activity. In humans the prevalence and pathogenesis of anaemia is poorly understood, though it is probably

due to multiple factors such as blood loss, renal failure, and ill-defined haemolytic processes.[282] Leptospirosis did not affect the functional ability of the bone marrow and no suppressive myelotoxic action was observed in bone marrow aspirated from 20 anaemic patients with leptospirosis.[17] Anaemia in patients with leptospirosis is predominantly a hyperregenerative nature with normoblastic haemopoiesis.[282] A contradictory report of erythroid hypoplasia associated with leptospirosis suggested that there may be a direct cytotoxic effect of *Leptospira* on early erythroid progenitors.[248]

Thrombocytopenia is a frequent finding in leptospirosis, though some studies reported no association with either clinical bleeding or DIC.[83,194] The mechanism of thrombocytopenia is not well understood and may be multi-factorial similar to the processes responsible for anaemia, perhaps including a direct toxic effect of the organism on the bone marrow,[194,248] non-immune platelet destruction as an effect of DIC,[108] immune-mediated causes of platelet destruction,[71,129] and increase consumption of platelets secondary to activation of vascular endothelium.[193,194]

1.3.8.2 Blood chemistry

1.3.8.2.1 Renal function tests

The incidence of proteinuria ranges from 40% to 80% in large case reports (**Table 1.3**). Casts can also be found on microscopic examination. Blood urea nitrogen and creatinine levels may rise rapidly during the early course of the illness. Hyperkalaemia from renal failure can occur, but hypokalaemia from the impairment of sodium transporters in the proximal tubules with spared function in the distal tubules is more common. Azotaemia in leptospirosis may occur as a result of pre-renal cause, importantly volume depletion, reflected by a high blood urea nitrogen, and a BUN:creatinine ratio of ≥20:1. This form of renal failure is readily reversible with proper fluid resuscitation.

A proportion of patients succumb to hypercatabolic acute renal failure with a high degree of azotaemia, and renal replacement therapy such as haemodialysis may be needed in addition to supportive treatment. A polyuric phase may develop after 10-18 days, and the serum creatinine value usually normalises 3-5 weeks after the onset of the illness.[277]

1.3.8.2.2 Liver function tests

Serum bilirubin levels may be very high and jaundice usually persists for around one month. In contrast the rise in transaminase levels are moderate. Elevation of alkaline phosphatase level usually occurs, reflecting the cholestatic rather than hepatocellular nature of the jaundice. These features are useful for differentiating leptospirosis from hepatitis of viral origin.[92]

1.3.8.3 Blood coagulation

1.2.8.3.1 Coagulation factors

Coagulation factors may be only slightly abnormal even in fatal cases associated with severe haemorrhage, suggesting that endothelial capillary damage rather than a hepatic or septic coagulopathy is the main haemorrhagic aetiology.[8,125]

1.3.8.3.2 Disseminated intravascular coagulation

Disseminated intravascular coagulation or DIC is a complex, systemic thrombohaemorrhagic, clinical syndrome, which is difficult to diagnose. There is no single diagnostic test to diagnose the condition. The DIC Scientific Subcommittee (SSC) of the International Society for Thrombosis and Haemostasis (ISTH) developed a consensus of the algorithm for the calculation of 'overt DIC score' using the platelet count, prothrombin time, fibrinogen, and D-dimer as parameters. A score cut-off of ≥ 5 had a 91% sensitivity and 97% specificity for a diagnosis of DIC.[269]

Controversy surrounds the rate of occurrence of DIC in leptospirosis, with evidence supporting both the presence[73,108] and absence[275,324] of DIC, using both animal and human pathological studies.

1.3.9 Laboratory diagnosis for leptospirosis

Due to the broad and nonspecific clinical presentation of leptospirosis, the diagnosis of leptospirosis on the basis of clinical manifestations is inaccurate. Laboratory tests are crucial for the confirmation of the diagnosis. There are three main modalities for the laboratory diagnosis of leptospirosis.

1.3.9.1 Isolation of leptospires

During the leptospiraemic phase, leptospires can be detected in the blood and CSF. The definitive diagnosis can be made by culture and isolation of *Leptospira* bacteria. The recommended media used for culture is Ellinghausen, McCullough, Johnson, and Harris (EMJH) medium, made to semi-solid condition. Culture is rarely used in the clinical setting because of the need for prolonged incubation, labour-intensiveness and low sensitivity. The culture should be maintained for at least 3 months before a negative result can be reported. No positive cultures occur among patients with a duration of illness longer than five days prior to admission.

Urine culture is less sensitive due to the nonviability of leptospires in the acid environment of urine. Furthermore, other bacteria in urine frequently overgrow the culture medium. Animal inoculation is not a routine practice for general diagnostic measures nowadays, but may be useful in research.

1.3.9.2 Serological tests

Several serological tests have been developed for the detection of *Leptospira* antibodies in humans. Antibody starts at the end of first week of illness and will peak 2-

3 weeks after onset. In general, blood for serology should be taken as soon as possible in the illness, and a second specimen should be taken a week to ten days later to compare with the admission result. A rise in antibody titre compared with the first sample reflects a recent active infection. The principle requires tests in which the antibody titre can be quantified, such as the microscopic agglutination test (MAT), indirect immunofluorescent assay (IFA), or enzyme-linked immunosorbent assay (ELISA). For other tests which provide only a qualitative result, positive or negative, the sensitivity of the test depends very much on the duration of illness before the test is performed. Examples include the immunochromatographic test (ICT), microcapsule agglutination test (MCAT), and latex agglutination test (LA).

The tests can be classified into two main categories:

1.3.9.2.1 Non serovar-specific tests

These tests can detect *Leptospira* antibodies in sera but do not give any information about the serogroups or serovars of the infecting *Leptospira* organism. Many tests are available in the market including the microcapsule agglutination test (MCAT), immune haemagglutination test (IHA), LA, complement fixation test (CF), ICT, ELISA and IFA. Some of these tests such as ELISA, IFA, ICT, may also be antibody type specific (IgG or IgM).

In general the diagnostic value of these tests in endemic areas is poor both in terms of sensitivity and specificity, and the tests are relatively expensive for developing country use.

1.3.9.2.2 Serovar-specific test

The most important test in this group is the MAT. Though this test shares many of the same problems as all serological tests for infectious diseases, the MAT currently remains the gold standard for the diagnosis of leptospirosis. The test itself is not easy to

perform, and is difficult to standardise between different laboratories. Standard strains of *Leptospira* representing each serogroup to cover the whole range of pathogenic serogroups need to be maintained in the laboratory. The laborious process of performing this test leads to it being available only in a few institutes, and it cannot routinely be performed in every hospital.

With the development of the new classifications of *Leptospira* by molecular methods, the importance and applications of these serovars and serogroups classification may well wane with time and the development of specific molecular diagnostic tests.[169] However, as antigen and DNA disappear quite quickly from the blood, it is likely that serological methods will continue to play some role in the diagnosis of leptospirosis for a long time to come.

1.3.9.3 Molecular methods

Polymerase chain reaction (PCR) methods for detection of *Leptospira* have been under development for nearly 20 years, and have been applied to serum, urine and other types of specimen. Several target genes have been evaluated, such as 16S ribosomal DNA[111], 23S rRNA[332], EcoRI fragment[139], and lipL32[48], with varying reported sensitivity and specificity. The tests are very useful for the detection of pathogenic *Leptospira* in animal urine, since urine shedding in animals occurs for a long period of time.[100] The sensitivity for detection of *Leptospira* in human serum is not satisfactory, due to the number of organisms in the specimens and the timing of specimen taken during the course of disease. Other PCR methods, nested PCR, nested PCR-restriction fragment length polymorphism (PCR-RFLP) and multiplex PCR have also been developed but they did not significantly increase sensitivity in clinical specimens.[79,155]

A new easy to perform and inexpensive molecular method using loop-mediated isothermal amplification (LAMP) targeting to LipL41 gene has also been developed for

the detection of pathogenic *Leptospira*. The lower limit of detection was approximately 100 copies.[175] The evaluation of the technique with human clinical samples has not yet been reported.

Though molecular methods of diagnosis need further development and are not yet practical for clinical use in human disease, they have been of some utility in the fields of epidemiology and strain identification. Real-time PCR may replace the conventional cross-aagglutination absorption test (CAAT) used for identification of *Leptospira*, which is very laborious and time-consuming.[246] Several methods such as multiple-locus variable number tandem-repeat analysis (MLVA), and multilocus sequence typing (MLST) can be a very powerful tool for outbreak investigation and epidemiological studies.[230,245,273]

1.3.10 Treatment

The specific treatment of leptospirosis is with antibiotics active against leptospires. These include penicillin, tetracycline, and the newer antibiotics such as doxycycline, azithromycin, and the cephalosporins. Two conflicting studies report the efficacy of penicillin against icteric leptospirosis compared with a control group with no antibiotic treatment. One shows halving of the fever clearance time with penicillin, and one show no difference between the two groups.[84,310] An evaluation of penicillin treatment in patients with leptospirosis and acute renal failure also shows no benefit in terms of mortality, duration of normalisation of renal function, bilirubin, platelet counts, length of hospital stay, and fever clearance time.[70] In contrast, the findings of the prevention of leptospiuria or a significant reduction of the duration of leptospiuria by the use of penicillin or doxycycline were consistent.[84,183,310] However, one meta-analysis concluded that the evidence suggests that penicillin may cause more good than harm.[104]

In a 2003 randomised study from Brazil of patients who presented after more than four days of illness, there was no mortality difference between penicillin treatment and no treatment.[63] Attention should be directed to earlier initiation of the treatment of leptospirosis to prevent the development of lethal complications.

In areas where leptospirosis is concomitantly endemic with other bacterial infections, the diagnosis cannot be made on the basis of clinical ground on admission. In severe disease empirical treatment to cover the potential causative agents is needed. Penicillin, though very efficacious against leptospirosis, is of limited use in other infections, especially those caused by Gram-negative bacteria. In vitro susceptibility of 11 serovars of *Leptospira* was confirmed against newer antibiotics, including cefotaxime, ceftriaxone, ampicillin-sulbactam, azithromycin, telithromycin, ciprofloxacin and moxifloxacin.[112] Two randomised controlled trials also proved that the efficacies of ceftriaxone[208] and cefotaxime[259] for the treatment of severe leptospirosis are at least equal to that of penicillin (and doxycyline for cefotaxime).

Doxycycline, but not penicillin, may be useful for short-term prophylaxis in high-risk settings[102,231,264] and for post-exposure prophylaxis following laboratory accidents.[101]

1.4 Scrub typhus

1.4.1 History of scrub typhus

The first record of a febrile illness thought to be scrub typhus (or tsutsugamushi disease in Japan) appeared in China in 313 A.D., described by Hong Ge in a clinical manual of the time called 'Zhouhofang', along with a description of the morphology of the vector mites. Later on in 610, Yuan-Fang Chao described the epidemiology, clinical course, and treatment of the disease in a poem, and in 1596, a well-known Chinese physician, Shi-Zhen Li, described the characteristics of the disease in a book entitled 'Ben Cao Gang Mu'.[93]

In 1879 Baelz and Kawakami first scientifically described 'flood fever' or 'tsutsugamushi disease', as an acute infection, but did not support the concept of mites as the disease vectors.[18] With this report as a turning point, many scientists in Japan began intensive studies of the disease. Increasingly the disease was believed to be caused by a virus transmitted through the bite of trombiculid mites in Japan.

Meanwhile in the other parts of the world fevers associated with a toxaemic stupor were known collectively by the term 'typhus', derived from the Greek 'typhos' which means 'stupor' or 'smoke' or 'haze'.[14] The most well-known variety of typhus, and also the most feared as it caused intermittent devastating epidemics, was 'epidemic typhus' (caused by *Rickettsia prowasekii*), which was proved by Charles Nicolle (who received the Nobel Prize in 1928) in 1909 to be transmitted by lice.[9] Various parts of the world also had different kinds of fever which were clearly 'typhus-like' in nature but occurred in quite different circumstances, sporadic rather than in sweeping epidemics, in warm countries rather than in temperate regions, in summer rather than in winter, and often associated with specific localities. Diseases were named according to the place or

the types of vector. This led to considerable confusion in the field of typhus research, and it took some time before other types of 'typhus' and 'typhoid' distinct from 'epidemic typhus' were correctly distinguished.

In Malaya in the 1920s and 1930s, Fletcher at the Institute of Medical Research saw many patients with features similar to endemic typhus, a disease initially described in the US and first described in Australia as murine typhus (caused by *Rickettsia typhi*).[103,110] It was characterised by acutely abrupt onset of fever with headache and vomiting, which usually suddenly disappeared after 14 days. Sera from these patients did not react with the wild (W) form of *Bacillus proteus* but reacted with *B. proteus* provided to Fletcher by Dr. Kingsbury, known later as the Kingsbury (K) strain of *B. proteus*. Fletcher called the disease 'tropical typhus' and found that in Malaya there were two forms of 'tropical typhus; the 'W' form and the 'K' form. The W form, or urban type, in which patient sera reacts with the W strain of X-19, usually occurs in town-dwellers, and the disease resembles the endemic typhus of America and Australia. K form or rural tropical typhus was a disease of the open country and not of the town. All the patients in Malaya were labourers working on plantations, mainly workers from the oil palm estate, mostly from the area where the jungle had been cleared to make way for oil palm trees since 1920. The name 'scrub typhus' was coined to describe the disease contracted in areas of 'scrub' growth following deforestation. Fletcher then concluded that the K form tropical or scrub typhus and endemic typhus are two distinct diseases.[97]

With the awareness of tsutsugamushi disease in Japan and other parts of Asia, Fletcher suggested that the two diseases had a similar aetiology and therefore may both be carried by trombiculae mites. He concluded however that the two diseases were distinct, as the fever in tsutsugamushi does not end abruptly at the end of second week as it does in scrub typhus, and he never found a primary sore or bubo in his scrub typhus

patients as had been described in tsutsugamushi disease. Furthermore, sera from tsutsugamushi patients react weakly with the Kingsbury strain, whereas very strong agglutination in a very high dilution occurs with sera from patients with scrub typhus.[97] Later Lewthwaite and Savoor demonstrated that scrub typhus, tsutsugamushi disease, the mite-typhus of Sumatra, and the coastal fever of North Queensland were one disease, varying locally in both severity of the disease and incidence of the eschar.[172-174] Firstly, they urged that the name 'scrub typhus' be dropped in favour of tsutsugamushi disease, but wartime usage firmly established the simple expression 'scrub typhus'.[14]

During World War II, the disease became a major problem among Allied forces and caused substantial morbidity and mortality. This high death toll and associated military importance led to research teams being set up by both sides to study the disease.

1.4.2 The *Rickettsia* and *Orientia*

Japanese researchers were able to finally identified the causative organism, which they called *Rickettsia tsutsugamushi*, by inoculating the pathogen into the anterior chamber of a rabbit eye.[192,201] The organism was found to be transmitted by mite vectors; usually trombiculid mites (*Leptotrombidium pallidum, L. akamushi, L. deliense* in Japan), but other types of trombiculid mites are able to transmit the organism in other areas.

In 1995 *R. tsutsugamushi* was reclassified in to a new genus, *Orientia*, due to the vast differences from other organisms in the genus *Rickettsia* in the morphological, chemical and genetic structures.[266] The organism is an obligate intracellular bacterium that propagates in the host cell cytoplasm by binary fission. Its size is approximately 0.5μm wide and 1.2-3.0μm long, which is slightly larger than that of *R. typhi* and *R. rickettsii*.[267]

O. tsutsugamushi displays antigenic variation, and many different antigenic types have been isolated, including the 3 majors types, Karp, Gilliam and Kato. The distribution of strains varies between the geographical areas.

1.4.3 The epidemiology of scrub typhus

Scrub typhus is endemic only in the Asia-Pacific region. As many as a million people may be infected each year, and around a billion people at risk for the disease.[311]

1.4.3.1 The geographical distribution of scrub typhus

The furthest north the disease has been reported is in the Primorye Region, Far East Russia.[289] Japan and Korea are very well known as the endemic areas of the disease. To the West, the disease has been reported in Kashmir, especially in a valley deep in Pakistan. There was an outbreak of 10 cases in the Torres Strait islands of Northern Australia in 2000-2001 after unusually heavy rain.[91] **Figure 1.8** shows the geographical areas where scrub typhus is endemic.

Figure 1.8 Map shows the geographic areas (grey) where scrub typhus is endemic.

Reports of cases in other parts of the world are imported cases among travellers who spent time in scrub typhus endemic areas.[81,312]

1.4.3.2 Seasonal incidence of scrub typhus

Seasonal variation in the incidence of scrub typhus invariably occurs in temperate regions, though is not obvious in tropical areas. In Japan, the distribution of disease varies according to the season (mainly temperature and humidity) and the areas.[229] The incidence of the disease in autumn (October and November) is higher in Korea than in Japan, while in the other seasons the incidences of outbreaks were much higher in Japan.[21] In the Pescadores Islands of Taiwan, the daily maximum temperature reaching 30°C was the predictor for the starting of epidemics of scrub typhus.[205]

1.4.3.3 Mode of transmission

Scrub typhus is a vector-borne disease transmitted by the bite of a larval-stage trombiculid mite (chigger). The habitat for these mites is brush and grass areas in conjunction with forested area. The mite larva feeds only once on a vertebrate, such as a mammal, before transforming to nymph and adult forms which are free-living in the soil and do not feed on blood. Infected adult mites can transmit *O. tsutsugamushi* to the next generation of mites via transovarial transmission. Humans are accidental hosts, and at the same time dead-end hosts as person-to-person transmission has never been reported.

Transmission through routes other than skin have been occasionally reported. A case of fatal scrub typhus contracted through working with *O. tsutsugamushi*–infected yolk sac material was reported in 1947.[286] In Korea, a similar case of laboratory-acquired scrub typhus was reported in a technician during the harvesting of L-929 cells using a tissue grinder, purification of *O. tsutsugamushi* by the Percoll density gradient centrifugation method, and *O. tsutsugamushi* cell membrane disruption using an

ultrasonicator without wearing a mask.[204] It was postulated that the respiratory tract was the route of acquisition of infection in both cases.

During the pre-antibiotic era, many famous doctors and researchers lost their lives after being accidentally or experimentally infected by *Rickettsia*. Indeed, the term '*Rickettsia*' was named after Howard Taylor Ricketts, who died in 1910 while investigating the aetiology of epidemic typhus, and *R. prowazekii* was named after Stanislaus von Prowazek who died of rickettsial infection in 1916 during an epidemic typhus investigation. Fatal laboratory acquired infection of scrub typhus was noted in a needle stick injury to a research assistant t MacFarlane Burnet, at the Walter & Eliza Hall Institute in Melbourne, Australia since 1943. (Unpublished data, Graves, S. personal communication) These cases serve to emphasise the need for employing biosafety level 3 (BSL3) facilities and practices for manipulating rickettsiae. Laboratories working with rickettsiae should have an effective system for reporting febrile illness in laboratory personnel, with access to appropriate antibiotic treatment in the early stage of disease.

1.4.4 Scrub typhus in Thailand

1.4.4.1 History of scrub typhus in Thailand

The first confirmed case of scrub typhus in Thailand was reported in 1952.[272] Cases of scrub typhus in Thailand have not been systematically studied until the last decade, though the epidemiological extent of the disease was thought to cover the whole country.[281] Sero-epidemiological surveys in rural Thai villages in 1982 and 1984 showed that the acquisition of antibody to *O. tsutsugamushi* occurred very early in life and the prevalence of the seropositivity was around 60 to 77%.[128,263]

Infection among vectors in Thailand was also high as 77% of 8 different chigger species were found to carry *O. tsutsugamushi*, with Karp the predominant strain.[239]

1.4.4.2 Current situation of scrub typhus in Thailand

According to the data from Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, the number of patients reported as having scrub typhus ranges from 1,100 to 4,000 cases per year, except for 2001 in which number of cases exceeded 5,000 cases. There has been a sustained increase in the number of patients over the past decade (**Figure 1.1**). This may due to the increased availability of cheap rapid diagnostic tests, or due to changes in the ecology of vector and reservoir (possibly related to climatic changes), or due to other unknown factors. Further investigations are really needed to address this situation. The age distribution of patients with scrub typhus in Thailand during 2004-2007 is shown in **Figure 1.9**.

Most of the patients are in the north and northeast of the country. There are more patients in the northern region than in the northeastern region (**Figure 1.10**). This differs from leptospirosis in which the majority of patients are in the northeast.

1.4.4.3 Seasonal incidence of scrub typhus in Thailand

There seems to be two peaks of incidence, a large peak in July and a small peak in October. The number of cases drops slightly during August and September (**Figure 1.11**). This may relate to agricultural activities in conjunction with temperature and humidity during each period of the year.

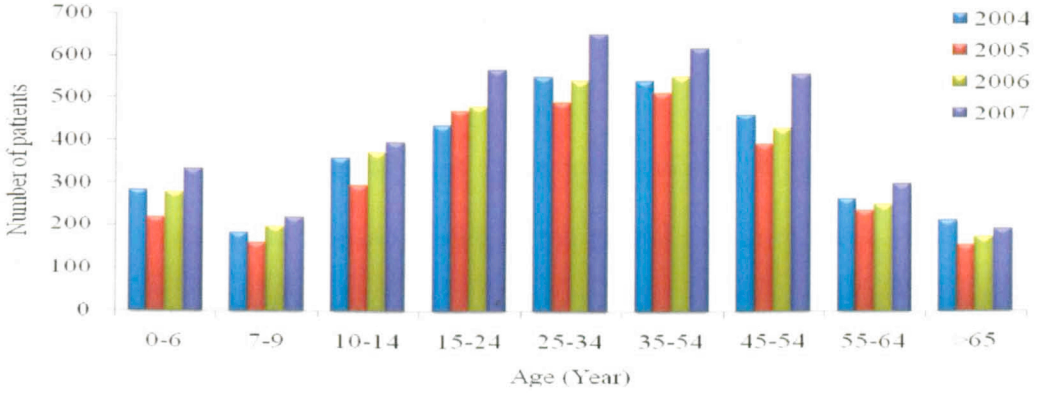

Figure 1.9 Age distribution of patients with scrub typhus in Thailand, 2004-2007

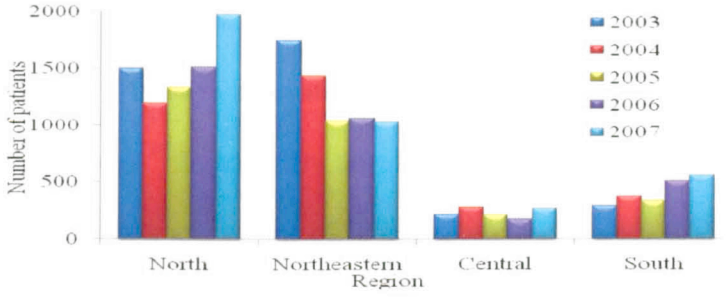

Figure 1.10 Distribution of patients with scrub typhus according to regions in Thailand, 2003-2007

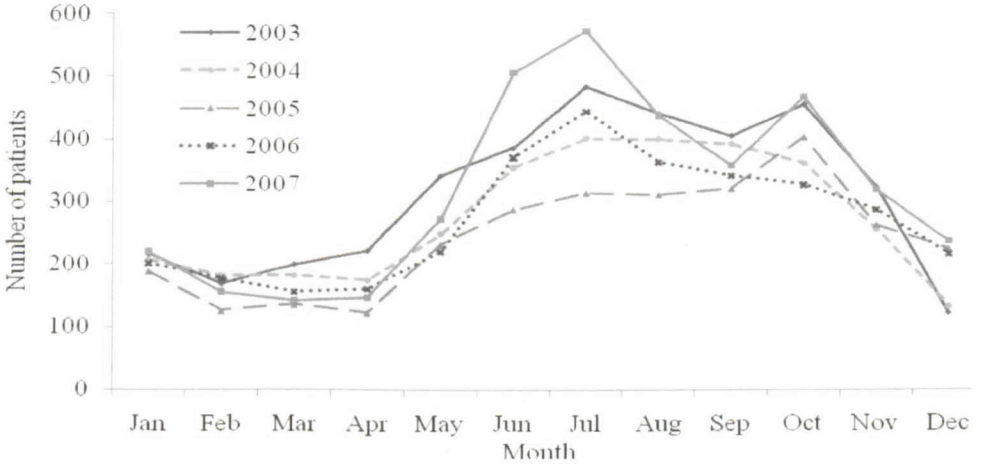

Figure 1.11 Number of patients with scrub typhus in Thailand per month from 2003-2007

(Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, http://epid.moph.go.th)

1.4.5 Pathogenesis

The chigger can bite on any part of the body, and often bites in areas where they are less easily detectable clinically, such as the genital region or axilla. The lesion is usually painless, and often goes unrecognised by patients.[149] Infection is thought to begin with localised multiplication at the site of the bite, followed by inflammation of the regional lymph nodes which drain the primary infection site, and then rickettsaemia

and invasion into endothelial cells resulting in a disseminated multiorgan vasculitis. In human experimental scrub typhus infection induced by a bite of infected mite, a febrile illness developed after 8-10 days incubation period. Bacteraemia was detected 1-3 days before the onset of fever.[238] In naturally acquired scrub typhus, the incubation period usually takes 6-20 days with an average of 10 days.[313]

The organism is an intracellular pathogen, which is taken up by phagocytosis escapes from the phagosome, replicates in the cytoplasm by binary fission, and then spreads to infect other neighbouring cells. The target cell of *O. tsutsugamushi* is poorly defined in humans, but is probably one or both of macrophages or endothelial cells. The basic histopathologic changes include disseminated perivasculitis and focal interstitial mononuclear infiltrations associated with oedema, suggesting that macrophages might be a more important target cell than the endothelium.[136]

The up-regulation of many cytokines and chemokines, such as lymphotactin, macrophage inflammatory proteins (MIP) 1 alpha/beta, MIP-2, monocyte chemoattractant protein 1, lymphotoxin beta, tumour necrosis factor (TNF)-α, interleukin (IL)-6, γ-interferon (IFN), transforming growth factor-β1, migration inhibition factor, etc., occur in animals with experimental scrub typhus infection.[153,330] In human cases, activation of IFN-γ, IL-18, IL-15, IL-12p40, TNF-α and cytotoxic lymphocytes (granzymes A and B, IFN-γ-inducible protein 10, and monokine induced by IFN-γ) was demonstrated in Thai patients.[53,74] Thus *O. tsutsugamushi* appears to be a strong inducer of chemokines and cytokines which may, by the attraction and activation of phagocytic leukocytes, significantly contribute to inflammation observed in scrub typhus.

1.4.6 Clinical manifestations

The clinical manifestations of scrub typhus vary from mild to severe, sometimes fatal symptoms. The systematic review of clinical manifestations of patients with scrub typhus is not as well established as in leptospirosis.

Prominent clinical presentations in scrub typhus consist of fever, headache and myalgia. Fever in scrub typhus usually rises suddenly and also disappears abruptly at around 2 weeks in untreated patients. Fever is usually accompanied by other constitutional symptoms. The prevalence of particular symptoms and signs in patients with scrub typhus reported from various parts of the world are shown in **Table 1.4**.

Table 1.4 Symptoms and signs of patients with scrub typhus in large case series

Symptoms and signs	Bourgeois[32]	Brown[35]	Tsay[283]	Ogawa[203]	Silpapojakul[242]	Sirisanthana[243]	Lee[165]	Phongmany[217]	Liu[176]
Number of patients	62	44	33	462	73	30	121	63	46
Study period	1975	Mar-Aug 1975	Aug 1993-Jul 1997	1998	Apr 1985-May 2002	Jan 2000-Dec 2001	2000-2004	Nov 2001-Oct 2003	Sep 2002-Dec 2004
Place	Pescadores, Taiwan	Central West Malaysia	Taiwan	Japan	Had Yai, Thailand	Chiang Mai, Thailand	Eastern Taiwan	Vientiane, Lao PDR	China
Mean Age (±SD) or median (range)	na	30 (11-57)	na	na	9 (3-14)§	§	na	31 (16-73)	42 (18-75)
Sex: male	†	32 (72%)	na	232 (50%)	64%	na	‡	40(63%)	25 (54%)
History of fever	100%	100%	100%	98%	100%	na	90%	na	na
Headache	85%	50%	21%	46%	31%	na	62%	95%	100%
Chill	79%	89%	39%	na	na	na	na	95%	67%
Myalgia	21%	na	na	16%	13%	na	na	na	100%
Malaise/arthralgia	/8%	na	na	63%	na	na	na	na	na
Conjunctival suffusion	25%	na	na	na	10%	33%	na	na	0
Anorexia	16%	na	na	na	na	na	na	na	85%
Nausea/vomiting	31%/10%	na	na	na	35%	na	na	62%/40%	41%
Abdominal pain	na	na	na	na	40%	na	na	35%	46%
Diarrhoea	2%	na	18%	na	28%	na	na	35%	na
Hepatomegaly/splenomegaly	-/2%	-/14%	na	3%/-	59%/18%	73%/23%	na	52%/15%	17%/-
Lymphadenopathy	63%	14%	33%	52%	23%	93%	11%	46%	61%
Rash	18%	0	21%	93%	11%	30%	22%	27%	76%
Eschar	100%	0	60%	87%	7%	68%	23%	52%	67%
Sore throat	41%	na	na	na	na	na	na	19%	na
Cough	19%	36%	24%	na	34%	na	na	38%	na
Dyspnoea	na	na	18%	na	na	na	na	na	na
Nuchal rigidity	na	na	na	na	6%	3%	na	12%	na
Retro-orbital pain	na	na	na	na	na	na	na	na	17%
Flushing face	na	na	na	na	na	na	na	na	46%

†Chinese military only ‡Survey by questionnaire retrospectively §In paediatrics
na: not available

Table 1.5 Laboratory findings and outcome of patients with scrub typhus in large case series

Laboratory findings	Bourgeois[32]	Tsay[283]	Ogawa[203]	Silpapojakul[242]	Phongmany[217]	Liu[176]
Place	Pescadores, Taiwan	Taiwan	Japan	Had Yai, Thailand	Vientiane, Lao PDR	China
Haematocrit	na	na	na	33 (24-41)	40 (23-50)	44 (36-45)
Platelet ($\times 10^9$/L)	na	na	na	162 (13-384)	200 (192-208)	143 (120-207)
White blood cell ($\times 10^9$/L)	na	na	na	9.5 (2.5-34.5)	11.8 (0.7-26.3)	6.7 (5.1-9.1)
Proteinuria	na	na	59%	na	na	28%
Creatinine (μmol/L)	na	na	na	na	106 (70-783)	na
AST (IU/L)	na	na	na	71 (17-159)	na	na
ALT (IU/L)	na	na	na	47 (6-197)	na	na
AP (IU/L)	na	na	na	83 (10-166)	175 (55-745)	na
Leukopenia (WBC <4000/mL)	na	19%	na	na	na	na
Leukocytosis (WBC >10000/mL)	na	34%	na	na	na	na
Thrombocytopenia	na	44%	na	19%	na	na
elevated creatinine	na	na	na	2%	na	na
elevated bilirubin	na	na	na	3%	8%	na
elevated AST	na	81%	87%	na	35%	na
elevated ALT	na	75%	77%	na	10%	na
DIC	na	na	14%	na	na	na
Mortality	1.6%	3%	na	na	1.5%	na

na: not available BUN: blood urea nitrogen AST: aspartate aminotransferase ALT: alanine aminotransferase

AP: alkaline phosphatase DIC: disseminated intravascular coagulation

Scrub typhus is an acute febrile illness characterised by focal or disseminated vasculitis and perivasculitis which may involve the lungs, heart, liver, spleen and central nervous system. The symptoms are usually mild and self-limiting, with spontaneous recovery after a few days. However, severe and protracted clinical illness can occur, and the disease may be fatal. The classic clinical description of scrub typhus usually comprises, but is not limited to, an eschar, regional lymphadenopathy, and maculopapular rash.

1.4.6.1 Constitutional symptoms

Headache, if present, is almost always severe and begins abruptly. The incidence of headache reported by various studies varied from 21% to 100%. It is less common in children (<50% in Thailand and 60% in a small retrospective review in Taiwan).[242,243,325] Headache symptoms may be atypical mimicking trigeminal neuralgia with frequent, severe, electric shock-like pain in one eye, the ipsilateral forehead, and scalp.[10]

Though myalgia usually presents accompanying with fever and headache in patients with scrub typhus, unlike in leptospirosis, histological studies on striated muscles have not been done in scrub typhus. Severe muscle tenderness as occurs in leptospirosis is also not obvious in scrub typhus, leading to one description in a text book that regards myalgia as a useful symptom to differentiate leptospirosis from scrub typhus.[313] However, life threatening rhabdomyolysis has been reported in an old patient with acute renal failure, suggesting this symptom may not be useful to distinguish the two diseases.[329]

Gastrointestinal symptoms often accompany the fever of scrub typhus, with abdominal pain, anorexia, nausea and vomiting are common. An endoscopic study in patients with scrub typhus who presented with gastrointestinal symptoms showed

varying degrees of mucosal changes along the upper gastrointestinal tract, ranging from non-specific hyperaemia, petechiael haemorrhage, superficial haemorrhage, erosion, to deep ulcer with active bleeding.[146]

1.4.6.2 Skin manifestation

1.4.6.2.1 Eschar

The portal of entry of *O. tsutsugamushi* is the chigger bite site. This site may develop later into an 'eschar'. An eschar was almost always found in patients with 'tsutsugamushi disease' described in Japan in the early days, but was completely absent in patients with 'scrub typhus' described in Malaya and Sumatra at the same periods.[97] The reported incidence of eschar has been very variable over the years (**Table 1.4**), though part of that variability may due to differences in the thoroughness with which physicians search for an eschar. Eschars may be difficult to observe on the dark skinned and usually appear in hidden areas; however a very low prevalence of eschar in scrub typhus has been noted in several countries,[181,236] and absence in some studies.[22,97]

Some types of mites, usually bird- and reptile-chiggers, can cause severe skin reactions with itching and dermatitis at the bite site. The itchy bumps occur because of allergic reactions to the saliva. This so-call 'scrub-itch' may be accompanied by a low-grade fever and secondary infections following scratching. These are distinct from the fever of scrub typhus caused by *O. tsutsugamushi*.[15]

The chigger bite can occur on any parts of the body, is usually painless, and is not usually recognised by the patients. However, some types of chiggers, especially *L. akamushi* and *L. palpale*, may cause a feeling that a tiny thorn has penetrated and stayed in the skin, causing pain when rubbed with clothing or the hand. The regions bitten are frequently the folds of soft skin in the axilla, upper leg, and abdominal skin, but bites are not limited to these regions, for example head, lower and upper extremities areas can

also be bitten.[136] Bites on the webs between the fingers, the popliteal area behind the knee, folds of skin of the genitalia, skin under the breasts and skin beneath areas that are constricted by clothing such as garters, belts, or brassieres have also been reported.[214] More than one infected mite can attack a patient, therefore more than one eschar can be found in a particular patient, but this occurs uncommonly.

A recent study from Korea reported a differential distribution of eschar in males and females. More than 90% of patients in Korea had eschar, including one patient with 3 eschars. Eschar is more common on the front in both sexes than on the back. The most common site of eschar was the area within 30 centimetres below the umbilicus in the male, and the front chest above the umbilicus in the female. The distribution of eschars is shown in **Figure 1.12**.[144]

The eschar is usually well developed by the time fever appears, and *O. tsutsugamushi* can be detected from the eschar lesion either by culture or PCR. Although there are variations in the eschar characteristics according to the species of trombiculid mite causing the dermatitis, it usually starts with a bright red papule with induration around the bite site. The peripheral area of the papule is not always well demarcated; subsequently a blister develops in the centre, and turns pustular with the enlargement of the surrounding erythematous area. Consequently, a small ulcer occurs with a blackish-brown scab in the centre, and then the surrounding erythematous area becomes darker. Ultimately, the black scab enlarges and dropped off leaving the underlying healed ulcer which will recover without scarring.[136] Atypical eschars can occur in warm and damp areas, in which a necrotic eschar is not formed; instead, an ulcer with a shallow, purulent base surrounded by a clear, erythematous band may be formed. These may complicate the diagnosis of scrub typhus and the lesion may be overlooked or ignored.[144]

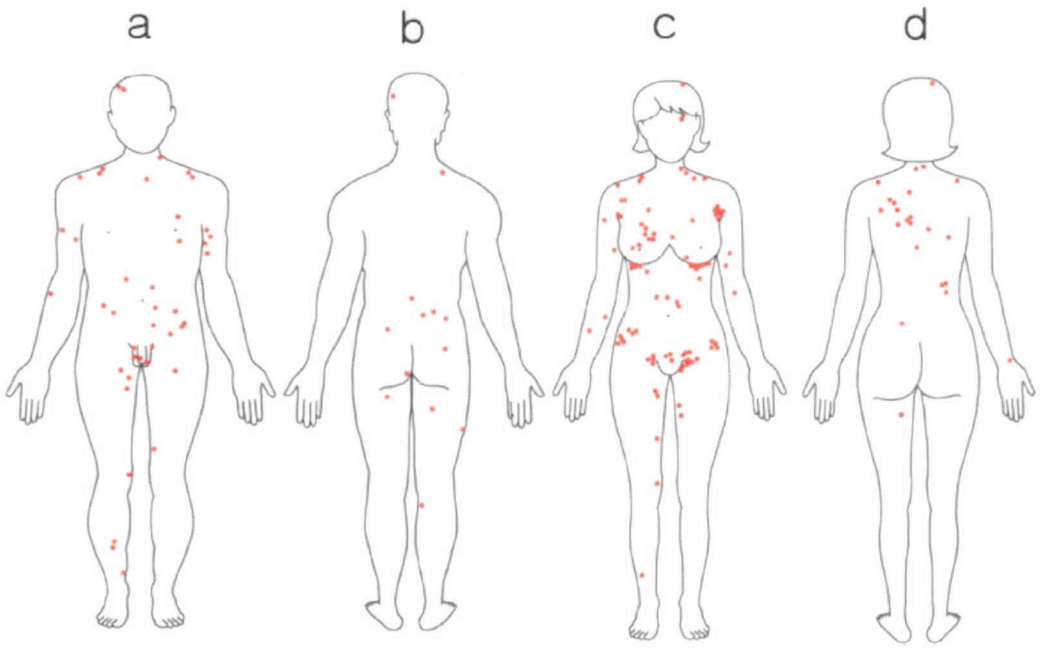

Figure 1.12 The distribution of eschar in scrub typhus patients
a) male front, b) male back, c) female front, d) female back [144]

1.4.6.2.2 Rash

A transient macular rash may appear at the end of the first week of illness. The rash usually occurs on the trunk and spreads peripherally. On the dark-skinned patients, the rash is often difficult to observe. The rash may begin on day 3 of illness and last for 3 to 7 days.[136]

1.4.6.3 Renal involvement

Renal involvement has been rarely mentioned in the papers, reviews, and text books concerning scrub typhus. It has been reported in sporadic cases from Taiwan and Korea.[51,113,329] Acute renal failure occurred as a complication in 9% of patients with scrub typhus in Taiwan,[283] and was found in 22% of patients with confirmed scrub typhus who presented with septic shock in Northeast Thailand.[274]

Acute renal failure associated with scrub typhus infection is not as rare as previously thought, especially in Thailand, Taiwan, and also in other endemic countries.

A renal pathology was reported in a Korean patient with scrub typhus and renal failure shows acute tubular necrosis due to the direct invasion of *O. tsutsugamushi*, demonstrated by immunohistochemical staining and electron microscopic examination.[142]

1.4.6.4 Liver involvement

Liver involvement is a prominent complication in patients with scrub typhus, though jaundice is not as obvious and as key a major finding as in 'Weil's disease'. The relationship between hepatic dysfunction and scrub typhus had been given little attention in the literature until the end of the last century. In three recent studies hepatic dysfunction was found up to 77%-96% of patients with scrub typhus.[45,115,322]

The most prominent abnormalities are rises in AST and ALT levels followed by increased alkaline phosphatase and bilirubin levels. In children, only 7.4% of patients in Thailand had jaundice, but up to 40% in adult patients in Taiwan.[45,115,322] A clinical feature resembling granulomatous hepatitis can be observed.[52] The pathology in the liver shows a dark-brown liver with diffuse whitish markings, features of non-specific reactive hepatitis including sinusoidal small lymphocytic infiltrations, mild disarray of hepatocytes and aggregation of T-lymphocytes and macrophages in the lobules.[130]

1.4.6.5 Cardiovascular involvement

Cardiac involvement during scrub typhus has been rarely reported, though myocarditis is marked as a common severe complication of the disease, especially in the pre-antibiotic era. Various ECG changes found in the acute febrile stage are non-specific and to be expected in acute infectious diseases that feature fever and increased cardiac output.[306] These findings include sinus brady- and tachycardia, flat or low T waves in the left precordial leads, tall peaked T waves in V2-4, T wave inversion in V3-4, ST segment elevation in V2, prominent U waves, incomplete right bundle branch

block, first degree atrioventricular blockage, prolonged Q-Tc interval, and other abnormalities. More dangerous arrhythmias can also occur; these may indicate that the prompt antibiotic treatment of scrub typhus can prevent the serious cardiac complications often reported in the pre-antibiotic era.[94,306]

1.4.6.5.1 Myocarditis

Myocarditis is one of the fatal complications of scrub typhus. The incidence varies between localities ranging from 3% in Taiwan to 17% in Sri Lanka.[223,283] The condition can also occur in children.[244,325]

An endomyocardial biopsy study in a patient with scrub typhus and myocarditis shows hydropic degeneration with loss of myofibril in myocardial cells, with mononuclear interstitial infiltrates and oedema. The capillary vessels in the cardiac tissue show endothelial cell swelling with many organisms in the cytoplasm.[328] The findings in the capillary vessels are consistent with the findings reported by other pathological studies in cardiac muscles and other organs such as liver, lung, brain, kidney, appendix, and skin.[285]

1.4.6.5.2 Pericarditis

This is a very uncommon manifestation of scrub typhus, with only one report in the literature.[44] A symptomatic pericarditis in a Boryong strain scrub typhus infection was diagnosed in a case with worsening dyspnoea, and presence of pericardial friction rub, cardiomegaly and pleural effusion on chest radiographs. Echocardiography revealed marked pericardial effusion and tamponade. The clinical course was improved considerably after pericardial drainage and doxycycline therapy.

1.4.6.5.3 Myocardial infarction

In patients with acute myocarditis, the symptoms, ECG changes, and cardiac enzyme findings may mimic acute myocardial infarction.[126] However, a genuine acute myocardial infarction may also occur. Whether this is a co-incidence or myocardial infarction occurs as a consequence of scrub typhus infection is unknown.[141]

1.4.6.6 Neurological involvement

Neurological involvement in patients with scrub typhus occurs commonly and was a prominent clinical feature in early descriptions of scrub typhus. In Fletcher's description of both scrub typhus and tsutsugamushi disease, he stated that both diseases commonly presented with '*the prominence and rapid development of nervous symptoms, the severe headache, the stuporose and irritable condition by day, the restlessness and delirium by night, the loss of knee-jerks and the deafness in the late stages, the tendency to bed-sores*'.[97] In the more recent literature the incidence of these manifestations varies between geographical areas, and it is rarely described as the most prominent feature of scrub typhus. In Thailand, neurological involvement was found in 3%-8% of children with scrub typhus[242,243] and 12.5% of adult.[241]

Headache is the most prominent neurological symptom as mentioned above. Confusion, apathy, and other mild personality changes are also common. Convulsions, tremors, delirium, nervousness and coma occur in a small proportion of patients.

1.4.6.6.1 Meningitis, meningoencephalitis and encephalitis

Meningitis in scrub typhus frequently occurs both in children and adults, and again the incidence varies from place to place. In general, patients usually present with severe headache and myalgia so it may be difficult to detect nuchal rigidity from meningitis same as in leptospirosis. Menigoencephalitis and encephalitis are less common, but not rare in scrub typhus. Patients may have alteration of consciousness, convulsion, and coma in severe cases. In Sri Lanka, a report of the features of

encephalitis in 4/19 patients with scrub typhus described high frequency coarse tremors of extremities associated with abnormal lateral head movements and rapid oscillations of the eyes in all directions.[222] All but one had complete recovery within 48 hours of doxycycline and/or intravenous chloramphenicol administration. One patient with concomitant pneumonitis and myocarditis died despite antibiotic treatment.

Pathological findings of the central nervous system in patients with scrub typhus show diffuse or focal mononuclear cell infiltrates in the leptomeninges and the presence of clusters of microglial cells and haemorrhages that are distributed throughout the brain substance. The CSF findings are of a mild mononuclear pleocytosis, increased protein level and normal sugar level, which are consistent with aseptic meningitis or viral meningitis. White cells in CSF may range from 0 to few hundred cells. Erythrocytes can also be found as a result of generalised vasculitis. Detection of *O. tsutsugamushi* antigen by PCR confirmed the invasion of organisms through the blood-brain barrier.[206]

1.4.6.6.2 Acute hearing loss

Hearing loss associated with scrub typhus infection is prominently mentioned in reviews of scrub typhus, but knowledge of the actual incidence and mechanism are very limited. The first mention of hearing abnormality in scrub typhus was published in 1929 with a further report in 1953.[97,195] Noad suggested that hearing loss is a very useful diagnostic clue for scrub typhus, since it occurred in one third of cases.[195] A long silence in the literature was broken recently with a report of an outbreak of scrub typhus in Sri Lanka in which 19% of patients had concomitant acute hearing loss.[222] Most of patients with this complication presented after 9-10 days of clinical illness, and all but one patient had a complete recovery from hearing loss. A patient with hearing loss who died also had pneumonitis, myocarditis and encephalitis as complications from severe

scrub typhus. Another two cases of acute hearing loss were reported from India confirming the presence of this neglected manifestation.[178]

The pathogenesis of acute hearing loss in patients with scrub typhus is not known. An audiographic study, being conducted in Vientiane, Lao PDR, will hopefully reveal the incidence and mechanism of hearing disorders occurring in this disease (Newton, P. personal communication).

1.4.6.6.3 Other uncommon neurological manifestations

Brachial plexus neuropathy was reported in a young man from Taiwan, who had a nearly complete recovery after medical treatment.[278] More serious and persistent neurological impairment can also occur: a US traveller returning from Thailand developed coma and multiorgan failure due to scrub typhus;[312] and an elderly Taiwanese man developed acute disseminated encephalomyelitis (ADEM), a monophasic demyelinating disease of the central nervous system typically occurring after infections or vaccinations.[49]

Encephalomyelitis with prominent focal neurologic signs has been described in a young woman.[140] Bilateral sixth and seventh nerve palsies, bilateral gaze evoked nystagmus, anarthria, dysphagia, quadriparaesis, and T1 sensory loss were found. Magnetic resonance image showed areas of signal hyperintensity in the dorsolateral pontomedullary region, bilaterally in the cerebellar peduncles, and in the cervical spinal cord.

1.4.6.7 Ocular involvement

Ocular involvement in scrub typhus is not as prominent as in leptospirosis. Conjunctival suffusion, which usually occurs in patients with leptospirosis, is hardly mentioned in the scrub typhus literature. However, in large case series reported in Taiwan and in children in Thailand, conjunctival suffusion was found in 25% and 33%

respectively.[32,243] Kato observed bilateral conjunctival injection, subconjunctival haemorrhage, and bilateral episcleral vessel dilatation in 4 patients with the 'new type' of tsutsugamushi disease or scrub typhus in Japan, which had never been noted in the more severe, 'old' or 'classic type' of tsutsugamushi disease.[132]

Bilateral conjunctival suffusion, flame-shaped retinal haemorrhage in one eye, and scattered retinal haemorrhages in the contralateral eye were reported in a Japanese old woman. Branch retinal vein occlusion and contralateral retinal haemorrhage was proven by fluorescein angiography.[191]

1.4.6.8 Lymphadenopathy

Lymph organs may appear as a primary site of organism invasion after skin inoculation, together with the blood stream spread. Lymphadenopathy is usually noted as one of the classic clinical presentations of scrub typhus. Human cases after experimental infected chigger bite clearly showed regional lymphadenopathy near the eschar sites in all cases.[238] The enlarged nodes are usually painful.

Generalised lymphadenopathy, not limited to the chigger bite site, is common as well. A study of chest and abdominal computed tomography in 11 and 19 patients with scrub typhus, axillary, hilar, mediastinal, and abdominal lymphadenopathy was found in 73%, 45%, 91%, and 47%, respectively. The lymph nodes were typically large and did not have necrotic centres.[127]

1.4.6.9 Splenomegaly

Spleens in patients with scrub typhus are usually enlarged and congested due to acute inflammation. Splenic infarction was also observed in 16% of patients.[127] In Southeast Asia, a palpable spleen was noted in 15%-23% of patients with scrub typhus.[217,242,243]

1.4.6.10 Bleeding diathesis

In general haemorrhagic tendencies are common in rickettsial infections, particularly Rocky Mountain spotted fever. In severe scrub typhus, haemorrhage was also claimed to be a prominent and critical complication, especially in pre-antibiotic days. DIC has long been noted as a cause of bleeding in scrub typhus, both in terms of pathological findings,[7] and clinical case descriptions.[18,23,137] After the introduction of antibiotics, several reports from Japan mentioned DIC found in patients, but it was not clear how the DIC has been defined. Among the reports of DIC, there is no mention of clinical bleeding among reported patients.[122,202] One study of 462 patients with scrub typhus in 1998, in Japan, reported DIC in 14% of patients using retrospective interviews of doctors in the hospital, with no questions related to clinical bleeding.[203] DIC was reported with no mentioned criteria in 8/18 (44%) patients with scrub typhus who came with septic shock.[274]

On the other hand, among patients with severe scrub typhus reported from Taiwan, no haemorrhagic complication occurred.[283] In a comparative study between 46 patients with scrub typhus and 49 patients with haemorrhagic fever with renal syndrome (HFRS) conducted in China there were no haemorrhagic manifestations in patients with scrub typhus.[176]

Upper gastrointestinal bleeding is the most common site of bleeding reported in patients with scrub typhus. It is a prominent feature among patients with ARDS.[297]

1.4.6.11 Unusual presentation

1.4.6.11.1 Acute appendicitis and acute acalculous cholecystitis

Patients with scrub typhus may present with severe abdominal pain mimicking peritonism from acute cholecystitis or acute appendicitis, which may lead to unnecessary operations.[323]

The gallbladder wall in patients with scrub typhus may be thickened due to acute vasculitis and perivasculitis causing subserosal oedema which can mimic acute cholecystitis. This condition can be distinguished from acute cholecystitis by the presence of gallbladder wall thickening in the absence of tense gallbladder distension.[127]

1.4.6.11.2 Relative bradycardia

Relative bradycardia, an unexpectedly low heart rate response for a given rise in body temperature, has been reported in a number of infections, for example typhoid fever, Legionnaires's disease, babesiosis, Q fever, pneumonia caused by *Chlamydia* spp., dengue fever, yellow fever, and viral haemorrhagic fevers. Relative bradycardia may also occur in 40-50% of the patients with scrub typhus.[13,95]

1.4.6.11.3 Scrub typhus and pregnancy

In endemic areas, scrub typhus can occur in pregnancy and though uncommon can result in serious consequences which occur both for the mother and fetus. Poor outcomes in newborns of mothers who are infected with scrub typhus during pregnancy include stillbirths, abortions, neonatal death,[182,218] neonatal scrub typhus. [182,258,298] In a recent report from Korea nine pregnant women with scrub typhus infections were successfully treated with a single dose azithromycin. All eight newborns that were able to be followed up were healthy, though one had an abnormally low birth weight.[147]

1.4.7 Pulmonary scrub typhus

Lung involvement in patients with scrub typhus may be very prominent and is a major cause of death in severe disease. The features include interstitial pneumonitis, interstitial oedema and haemorrhage, caused by vasculitis.

1.4.7.1 Respiratory symptoms and signs

The proportion of patients presenting with the respiratory symptoms is very variable, with the occurrence of cough, for example, ranging from 19% to 64% (**Table 1.4**). Another unexpected symptom for scrub typhus is a sore throat, which occurs quite commonly in Taiwanese patients,[23,32] and in one-fifth of Lao patients.[217] Sometimes the sore throat may be severe and mimic acute pharyngotonsilitis and lead to the delay of proper treatment for scrub typhus.[23] In a series of chest radiographic studies, chest symptoms was found in more than 60%, and haemoptysis occurred in 3/84 patients.[46]

1.4.7.2 Chest radiographs

Chest radiograph abnormalities range from 59%-72% in Korea and 65% in Thailand.[46,56,249] The most common abnormal findings are bilateral diffuse areas of reticulonodular opacity, hilar lymph node enlargement, and septal lines. Airspace consolidation is relatively uncommon and generally appears in the lower zone of both lungs. Unilateral or bilateral hilar lymphadenopathy and pleural effusion are found in one-forth, and 12%-43% of patient in Korea, respectively,[56,249] whereas parenchymal consolidation and hilar lymphadenopathy were found in less than 2% of Thai patients.[46] Cardiomegaly on chest radiographs was observed in 13%-30% of patients, and was usually reversible.[46,56]

1.4.7.3 Severe pulmonary involvement

Respiratory failure is a severe complication of scrub typhus and a leading cause of death. Symptoms start with tachypnoea and progress to dyspnoea, then the patient becomes cyanotic, and full-blown adult respiratory distress syndrome may develop. Severe pulmonary scrub typhus can mimic other fatal conditions or diseases such as

severe acute respiratory syndrome (SARS), haemorrhagic fever, HFRS, and severe pulmonary leptospirosis.[297]

Adult respiratory distress syndrome (ARDS) can be defined by the criteria set by the American-European Consensus Committee: 1) acute onset timing; 2) chest radiograph showing bilateral lung infiltrates; 3) severe hypoxia with a partial pressure of arterial oxygen to fraction of inspired oxygen ratio (PaO_2/FiO_2) ≤200mmHg, regardless of the level of positive end-expiratory pressure (PEEP); and 4) no clinical evidence of increased left arterial pressure with a pulmonary arterial wedge pressure ≤18mmHg.[25] ARDS was found in 11% of scrub typhus patients in Taiwan. In multivariable analysis, a low level of albumin, a prolonged prothrombin time, and duration of illness before the commencement of appropriate antibiotic are independent risk factors for developing ARDS.[297] In another study from the same country the identifiable risk factors for ARDS were older age, thrombocytopenia and the presence of early pneumonitis.[284]

An autopsy on a patient who died from acute respiratory distress syndrome and progressive respiratory failure from scrub typhus showed diffuse alveolar damage without evidence of vasculitis and no demonstrable organisms in the lung tissue.[211] A report from Taiwan of autopsies on two patients died from ARDS and scrub typhus showed similar diffuse alveolar damage and hyaline membrane formation, but also an interstitial pneumonitis with infiltration of inflammatory cells and the presence on immunohistochemical staining of *O. tsutsugamushi* antigen depositions in the endothelial cells reflecting direct endothelial cell invasion by the organism.[114]

1.4.8 Laboratory findings in scrub typhus

1.4.8.1 Complete blood count

Very little information concerning the features of the complete blood count in patients with scrub typhus is described in the literature. Anaemia is a prominent finding

in patients with other typhus group infections and other rickettsioses, and can be found in nearly half of the patients. In large scrub typhus case series, haematocrit level were normal in adult patients, but low in Thai children.[176,217,242]

White blood cell counts in patients with scrub typhus can be normal, high or low. The white blood count was normal (WBC 4,000-9,000cells/mL) in about half of the patients (47%-55%), and leucocytosis (WBC >9,000cells/mL) and leucopenia (WBC <4,000cells/mL) occurred in 19%-34% and 19%-26% respectively.[203,283]

Thrombocytopenia, prominent feature in leptospirosis, occurs to a lesser extent in scrub typhus but is still present in 19%-44%.[242,274,283]

1.4.8.2 Blood chemistry

Patients with scrub typhus usually have normal blood urea nitrogen and creatinine levels. However, renal failure and elevated creatinine levels may be associated with mortality in patients with scrub typhus.[293] Electrolytes and albumin levels are scarcely mentioned in the scrub typhus literature. Albumin levels may be low, but are not significantly different between patients with and without ARDS.[297] Surprisingly, in the same study, multivariable analysis conversely shows that a low albumin level is an independent risk factor for ARDS.

The bilirubin level is usually normal or slightly elevated, though very high levels of bilirubin can occur. As mentioned earlier in the clinical section, hyperbilirubinaemia can be found up to 40% of patients. The majority (70%->95%) of patients have high AST and ALT levels.[45,115,322] The alkaline phosphatase may be high in around 50% of patients. The levels of AST and ALT are usually 2-5 times elevated from the upper limit of normal range, but very high levels mimicking viral hepatitis can be encountered.[322]

Coagulation studies have never been properly done in patients with scrub typhus, though DIC is claimed to be a major cause of death in many patients.

1.4.9 Laboratory diagnosis of scrub typhus

Except for the presence of the eschar, clinical history and symptoms and signs are not specific for scrub typhus. A laboratory diagnostic test is needed for the confirmation of infection. As with leptospirosis and other bacterial infections, there are several types of tests.

1.4.9.1 Isolation of *Orientia tsutsugamushi*

Isolation of the causative organism is the most definitive and gold standard method for almost all of the bacterial infections. For all *Rickettsiaceae*, the difficulty for isolation is the methodology. *Rickettsiaceae*, including *O. tsutsugamushi*, are obligate intracellular bacteria which require a living host cell to grow in, either in an animal or in cell culture. The most commonly used method for isolation of *O. tsutsugamushi* is in cell culture, which need infrastructures and expertise, and laboratories with BSL3 facilities. So the technique is not routinely used in clinical diagnostic practice.

1.4.9.2 Serological tests

Similar to leptospirosis, serological tests are the most widely used tools for the diagnosis of scrub typhus, though the accuracy and interpretation of these tests are still major issues. Several serological tests were developed for the diagnosis of scrub typhus. The tests which are most widely used are as follows:

1.4.9.2.1 Weil-Felix test

This is a very long-standing test developed to detect patients with epidemic and endemic typhus, using the cross-reactivity of patient serum to *P. mirabilis* X-19 strain, as mentioned in the historical section. For detecting scrub typhus, patients may show cross-reactivity to X-K strain instead. This was the only test available in 1925-1926, and was used by Fletcher for the investigation of scrub typhus in Malaya. It may remain the

most widely used test for the diagnosis of 'typhus group rickettsioses', including scrub typhus, though many recent studies indicate that it lacks sensitivity and specificity and it is no longer recommended for the diagnostic purposes.[156,179,220,317]

1.4.9.2.2 Immunoperoxidase assay (IIP)

The test can differentiate IgG and IgM, but cannot differentiate the serotypes of infection. This method can be used as a gold standard for the diagnosis of scrub typhus in some regions. The technique uses peroxidase in the reaction as the indicator of positive result, so there is no need for an expensive fluorescence microscope and the tested slide can be kept permanently.

1.4.9.2.3 Indirect immunofluorescence assay (IFA)

IFA is most widely used and accepted as a gold standard for the diagnosis of scrub typhus. The method requires the use of fluorescence microscope which is relatively expensive and is usually not available in rural areas. The technique is similar to IIP, but using fluorescein dye as the indicator, so the tested slide cannot be kept for future checking.

Despite the gold standard use of IIP and IFA, the interpretations of the test and the standardisation of the methods used are confusing. For the presumptive diagnosis of active disease, the four-fold rising of the pair individual sera collected at least 1 week apart, or a cut-off titre of 1:400 in a single serum sample are recommended.[38] For the determination of the prevalence of disease in community, non-standardised cut-off values are used, so it is a difficult judgement whether the differences between studies and sites reflect actual differences in the population or are due to differences in cut-off.[29]

1.4.9.2.4 Rapid serological tests

There are a number of 'point of care' tests available for diagnosing scrub typhus, though some tests may not be 'rapid' or 'bed-side', since they may take hours instead of minutes to perform and may need many steps requiring laboratory equipments. The immunochromatographic flow assay is the test of choice in this group considering 'time' and 'ease'. The test was developed from the enzyme-linked immunosorbent assay (ELISA) technique, coding on a nitrocellulose strip and needing only eye-reading. The test can also differentiate IgG and IgM.[54,315]

Other tests which have been developed and evaluated include complement fixation (CF), ELISA, latex agglutination (LA), and dot-blot immunoassay. **Table 1.6** shows examples of sensitivities and specificities of various serological tests published in the literature.

1.4.9.3 Molecular-based diagnosis

Polymerase chain reaction (PCR) assays to amplify parts of the 56kDa, 47kDa, and 16s rRNA genes of *O. tsutsugamushi* have been developed and evaluated. However, the sensitivity of these tests in clinical samples from the endemic areas needs to be improved. An evaluation of 16S rRNA and 56kDa genes PCR yielded 45% and 29% sensitivity, respectively.[250] An improvement of PCR by the modification of conventional PCR to nested PCR of 56kDa gene gave a better result in the clinical evaluation. Using IFA as a gold standard test, nested PCR yielded 82% sensitivity and 100% specificity in 135 patients with suspected scrub typhus.[145]

The PCR method can also be used to detect organisms in the eschar lesion.[143] This may be useful in differentiating other rickettsioses from scrub typhus and for research purposes, but it is rarely of utility in aiding clinical judgement in areas such as Southeast Asia where other rickettsioses with eschars are very rare. Therefore, the

eschar is considered as a pathognomonic sign of scrub typhus, and prompt treatment should be commenced without further delay.

Other molecular techniques such as real time PCR are under development and evaluation. Recently, a simplified molecular method, loop-mediated isothermal PCR assay (LAMP) targeting the groEL gene encoding *O. tsutsugamushi* heat shock protein, has been evaluated in a small number of patients with scrub typhus.[210]

Table 1.6 Sensitivity and specificity of serological tests for scrub typhus diagnosis

Studies	Year Place	Sample (No of patients)	Standard serology			Rapid serological tests					
			WF 1:160	IFA IgM ≥1:400	IIP ≥ 1:400	LA	ELISA	Dip-S-Ticks	Multitest	Dot EIA	LF IgM
Pradutkanchana[220]	1997 Thailand	192 (129)	52.1 93.3	Gold standard	90.6 100			94.0 98.7			
Land[160]	2000 Australia	148			Gold standard		86 84				
Ching[54]	1976-77 Taiwan	321 (85)		Gold standard							74.0 99.0
Coleman[62]	2002 Thailand	350		85.1 98.3	Gold standard		94.0 92.0	60.3 97.4			82.6 93.4
Jang[123]	2003 Korea	176		≥1:80 Gold			100 99.0				
Watt[304]	2005 Thailand	310			Gold standard				47.0 -		
Wongchotigul[317]	2005 Thailand	209	47.3 92.6	Gold standard		89.1 98.2					
Prakash[221]	2006 India	-	43.5 -	Gold standard			86.5 -			100 -	

WF: Weil-Felix test IFA: indirect immunofluorescence assay IIP: immunoperoxidase test LA: latex agglutination test

ELISA: enzyme-linked immunosorbent test EIA: enzyme immunoassay LF: lateral flowFigures in the cells under each test represent the sensitivity in the upper row and specificity in the lower row.

1.4.10 Treatment

O. tsutsugamushi is very sensitive to antibiotics, and the response to the treatment is very prompt. Patients generally become afebrile within 24 to 36 hours of starting antibiotics. The early administration of antibiotics can abort symptoms and prevent the progression of severe complications. The recommended treatment of choice is a seven day course of oral doxycycline, 100mg twice daily, in uncomplicated cases, and intravenous doxycycline at the same dose, or chloramphenicol, 500mg qid, in severely ill patients.

Single doses of 200mg doxycycline, 500mg azithromycin or roxithromycin have been observed to successfully treat patients with scrub typhus.[36,55,91,147,163,307] A randomised, controlled trial may be needed to evaluate carefully the efficacy of single dose use of doxycycline and roxithromycin. Three-day course and a single dose azithromycin have been evaluated in Thailand and Korea, respectively. Both regimens show similar efficacy comparing with 1-week course of doxycycline. This drug can be used safely in pregnancy and childhood.[148,216]

Ciprofloxacin and cefuroxime failed to treat scrub typhus in pregnancy and resulted in fetal loss.[147] Fever subsides slowly in patients treated with ciprofloxacin, and unfavourable outcomes occur more often than with doxycycline.[181]

Drug resistant *O. tsutsugamushi* has been reported from Northern Thailand, and delayed clinical responses to treatment were observed.[303] Rifampicin is more effective than doxycycline for the treatment of scrub typhus in areas of emerging drug resistance *O. tsutsugamushi*.[308] However, this drug is very crucial for the treatment of tuberculosis, and incautious use may induce multidrug resistance tuberculosis which is a more complicated clinical problem than scrub typhus. Doxycycline-resistant strains of *O. tsutsugamushi* are usually sensitive *in vitro* to azithromycin.[254]

Chemoprophylaxis using weekly dosing of 200mg doxycycline starting 3 days before exposure until 6 weeks after exposure can prevent scrub typhus infection in 89% of volunteers, but minor self-limiting symptoms may occur 10 days after cessation of the prophylaxis.[288]

1.4.11 Outcome of scrub typhus

In the pre-antibiotic era and during the World War periods, scrub typhus killed many people and was a major public health and military problem. The disease in some parts of Japan carried a very high mortality rate before the advent of chemotherapy, exceeding 60% in one area.[137,265] On the other hand, in Pescadores, Taiwan, the mortality of scrub typhus was only 3%,[137,321] and in the outbreak in Malaya reported by Fletcher, there were only two deaths.[97] Following the introduction of effective antibiotics the disease is immediately curable, and if treated early the clinical symptoms are mild. However, when the disease is not suspected and patients receive inadequate treatment, the severe disease can develop and death can occur.

1.5 Co-infections

1.5.1 Concomitant leptospirosis and scrub typhus infections

Leptospirosis and scrub typhus are endemic in the same areas of Southeast Asia, particularly in northeastern Thailand. Reports of coinfections with these two disease entities are surprisingly rare. A Thai rice farmer from Northeast Thailand presented with typical features of Weil's disease, the severe form of leptospirosis. A cigarette burn-like lesion, characteristic of a scrub typhus eschar was noted on physical examination; this raised awareness of the coinfections of leptospirosis and scrub typhus in Thailand.[305]

A study conducted in 1997 confirmed the existence of concomitant infections in 9/12 (41%) of patients with laboratory-confirmed leptospirosis. Patients with concomitant infections had significantly higher platelet counts, and significantly lower bilirubin and creatinine levels. The other clinical features were not significantly different, except for the presence of eschar occurring in the mixed infections only. Two patients with mixed infections deteriorated after penicillin treatment for presumed leptospirosis diagnosis, and developed respiratory failure. The clinical improvement occurred after changing the antibiotic to intravenous chloramphenicol in one patient, but the other died from adult respiratory distress syndrome despite very high dose intravenous penicillin.[305]

A randomised controlled trial comparing the efficacy of intravenous penicillin, doxycycline and cefotaxime for the treatment of severe leptospirosis was conducted in a multi-centre setting in Northeast Thailand. Despite the experienced physicians in the areas who see tens of leptospirosis cases a month, the recruited patients had leptospirosis in 256/520 (49.2%), 71 (13.7%) patients had scrub typhus infection and 62 (11.9%) had mixed leptospirosis and scrub typhus infections. The efficacy of penicillin for the treatment of patients who were infected with scrub typhus was clearly inferior to

that of doxycycline or cefotaxime. The rate of treatment failure in penicillin group was 32.5% compared to 10.6% and 11.1% in doxycycline and cefotaxime groups, respectively.[259]

Recent reports from Taiwan emphasised the existence of concomitant leptospirosis and scrub typhus infections in this region.[50,162,300] Patients with coinfections in this report had the mixed features of clinical manifestations of both leptospirosis and scrub typhus, for example jaundice together with marked elevations of several liver enzymes.[162] Uncommon manifestations of both diseases, acute pancreatitis and acute acalculous cholecystitis, were reported in a patient with co-infections who also had acute renal failure.[300]

These reflect the importance of the accurate diagnosis measures for both diseases. In general doxycycline, which is generally used in uncomplicated cases of leptospirosis, is effective against both leptospirosis and is the drug of choice for treating scrub typhus. So in uncomplicated cases, the treatment is not a problem even though the definitive diagnosis may not be obtained. The problems may arise when the patients are severely ill, due to several factors. In severe complicated cases, either leptospirosis or scrub typhus, patients cannot take drugs or food orally, and parenteral treatment/feeding is needed. Intravenous doxycycline is not available in many countries, so the drug of choice for both diseases cannot be used in this instance. In severe disease, the clinical manifestations of leptospirosis, scrub typhus, or mixed infection may mimic other Gram-negative sepsis, so the choice for empirical treatment in this setting may be complicated by this fact. The use of a third generation cephalosporin such as cefotaxime and ceftriaxone can treat both Gram negative sepsis and leptospirosis, but this is not recommended for the treatment of scrub typhus or other rickettsioses due to the unsatisfied in vitro activity. Whether combination antibiotic treatment with either oral

doxycycline or intravenous chloramphenicol should be used depends on the probability of the scrub typhus diagnosis in a particular patient.

1.5.2 Other co-infections

Both scrub typhus and Human Immunodeficiency Virus (HIV) infection are prevalent in northern Thailand. Co-infection with the HIV occurred in 16% of patients in Chiang Rai province, but does not affect the clinical manifestations of *O. tsutsugamushi* infection, unlike the effect on other intracellular pathogens, such as tuberculosis, leishmaniasis and trypanosomiasis.[131]

Co-infection with malaria and leptospirosis has also been reported from Thai-Myanmar border, an area endemic for both diseases.[318] Antimalarial drugs, apart from doxycycline, are not active against *Leptospira*. Therefore, treatment focusing on malaria could result in a delay in treatment for leptospirosis and the possible development of serious complications.[189]

1.6 Aims of this dissertation

Leptospirosis and scrub typhus are major public health problems in Southeast Asia, especially northeast Thailand. Both diseases are endemic within the same geographical areas, share similar clinical manifestations and co-infections can occur leading to the diagnostic and management difficulties. The diseases have never been compared together, except for two small reports of co-infections from Thailand and Taiwan, despite our comprehensive knowledge of each individual disease. The specific aims for this study were to:

1. Describe the epidemiology of acute febrile illness in Udon Thani, northeast Thailand.
2. Describe the detailed clinical manifestations, laboratory findings, and outcomes of patients with leptospirosis.
3. Describe the detailed clinical manifestations, laboratory findings, and outcomes of patients with scrub typhus.
4. Compare the clinical manifestations, laboratory findings, and outcomes between leptospirosis and scrub typhus, and develop a simple scoring system to predict and differentiate between the two diseases.

Chapter II: Materials and Methods

2.1 Patients recruitment

A 26-month prospective study of acute febrile illness was conducted between October 2000 and December 2002 at Udon Thani Regional Hospital, a 1,000 bed regional hospital, northeast Thailand. Adult patients were identified from examination of daily admission logs performed during twice daily ward rounds of 5 medical wards and the intensive care unit and through discussion with treating clinicians. Patients were enrolled into the study after they had given written informed consent. The study protocol was approved by the Ethical Review Sub-committee of the Ministry of Public Health, Royal Government of Thailand.

2.1.1 Patients

The study population consisted of consecutive adult patients admitted with a history of acute febrile illness and non-specific systemic signs and symptoms or a suspected diagnosis of leptospirosis and/or scrub typhus.

2.1.1.1 Inclusion criteria

1. Patients with a history of acute fever (onset of less than 14 days)
2. Patients with non-specific systemic signs and symptoms or a suspected leptospirosis and/or scrub typhus based on:

- History of contact with suspected contaminated reservoir such as rice field or other sources of potentially contaminated water, and
- History of fever in conjunction with one or more of the following: malaise, myalgia, photophobia, headache, eye redness or subconjunctival haemorrhage, jaundice, oliguria or anuria, or

- Presence of an eschar lesion or history of recent chigger bite

3. Age ≥14 years

4. Willingness to participate in the study and written informed consent obtained from the patient

2.1.1.2 Exclusion criteria

If any of the following information was available from another hospital or from the clinic prior to admission, the patients were excluded from the study.

1. Patients with known positive asexual form of *Plasmodium* spp. on blood smear

2. Patients with other known definable infections such as acute suppurative pharyngotonsillitis, bacterial pneumonia, liver or splenic abscesses and urinary tract infection, etc.

2.1.2 Methods

After enrolment, details of clinical illness and physical examination were recorded into a clinical record form. Blood was taken on admission for aerobic blood culture, *Leptospira* culture, serological tests, coagulation tests and routine laboratory tests, as described below. A chest radiograph was taken in all patients and other radiographic studies were performed as clinically indicated. Other clinical specimens were collected for biochemical or microbiological tests according to clinical need, including cerebrospinal fluid (CSF) examination in patients with meningism. All aspects of medical care of the patients were undertaken by the admitting hospital physicians. A further serum sample was taken on the day of discharge or day 4 (whichever was the longer), and again on day 7 if still an in-patient. These samples were used as convalescent sera in the event that patients were lost to follow up. Patients were

asked to return to clinic to determine outcome and to obtain a convalescent serum 2 weeks after discharge.

2.2 Data collection

Details of exposure, clinical illness and physical examination were recorded onto a clinical record form. Details of in-hospital clinical progression, treatment and outcome were also recorded. The clinical record form is shown in **Appendix A** and contains details of the following factors.

2.2.1 History of exposure

This included patient occupation, recent travel, housing and working environment, contact with animals and water, and history of insect or animal bites. The type of water reservoir and duration of contact were recorded. Time of contact was recorded in relation to timing of onset of illness.

2.2.2 History of illness and physical examination

Onset of illness, symptoms including details of fever, headache, and other related clinical symptoms were recorded. All clinical symptoms and signs listed by WHO as clinical criteria for diagnosis of leptospirosis were included, as follows: type of onset of headache (sudden or insidious), presence of fever $\geq$ 39°C, conjunctival suffusion, meningism, muscle pain especially in the calf muscles, and jaundice.[92]

2.2.3 Clinical course during hospitalisation

Progression of disease during hospitalisation was recorded in terms of improvement or deterioration of clinical sign and symptoms, complications from disease and/or treatment, procedures or operations needed such as venesection or central line monitoring, respiratory support and cardiopulmonary resuscitation.

2.2.4 Treatment and outcomes

The details of treatment given to the patients were recorded, including antibiotics, antipyretics, inotropic drugs, steroids, blood and/or blood products and renal replacement therapy. Response to treatment was recorded in terms of fever clearance time and duration of hospitalisation. Outcome of treatment was recorded as improved or died.

2.3 Laboratory investigations

2.3.1 Diagnostic investigations

2.3.1.1 Serological tests

Blood was taken on the day of admission (7mL), days 4 and 7 after admission date (or day of discharge), and on day 14 (follow up), from which serum was collected and stored at -20^{O}C.

2.3.1.1.1 Leptospirosis

2.3.1.1.1.1 Microscopic agglutination test (MAT)

MAT was performed by the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Queensland Health Scientific Services, Australia (Prof Lee Smythe) as previously described,[4] using a panel of antigens representing both ubiquitous and locally prevalent serovars and representing 20 serogroups. In brief, the reference strains were sub-cultured and diluted 1:2. The prepared antigens were mixed with 2-fold serial dilutions of serum. The degree of agglutination was evaluated under dark-field microscopic examination. The endpoint was defined as the dilution of serum at which 50% agglutination occurred compared with a control culture diluted 1:2.

2.3.1.1.2 Scrub typhus

2.3.1.1.2.1 Indirect immunofluorescent antibody (IFA) assay for scrub typhus

IFA was used for the diagnosis of scrub typhus. The test was performed at the Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand. This was performed on paired (acute and convalescent) sera using established methodology.[34] In summary, mixed antigens of three strains of *O. tsutsugamushi* (serotypes Karp, Kato and Gilliam) were spotted onto a glass slide made by the National Research Institute of Health (NIH), Ministry of Public Health, Thailand. Initial screening was performed using a serum dilution titre of 1:50 for both acute and convalescent sera. Samples that were positive at this titre were further tested using 2-fold serial serum dilutions ranging from 1:100 to 1:6,400. IgM and IgG antibodies specific to scrub typhus antigens were detected by the addition of fluorescein isothiocyanate (FITC)-labelled anti-human IgM and IgG antibodies, respectively. Antibody binding was determined using a fluorescent microscope (Olympus BX50; Olympus Corporation, Japan). Known positive and negative control sera were run with each experiment.

2.3.1.1.3 Murine typhus and other rickettsial infections

2.3.1.1.3.1 Indirect immunofluorescent antibody (IFA) assay for murine typhus

IFA for murine typhus was performed by the Division of Infectious Disease, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand. This was performed on paired (acute and convalescent) sera using established methodology.[34] In summary, *R. typhi* antigens were spotted onto a glass slide made by the National Research Institute of Health (NIH), Ministry of Public Health, Thailand. Initial screening was performed using a serum dilution titre of 1:50 for both acute and convalescent sera. Samples that were positive at this were further tested using 2-fold serial serum dilutions from 1:100 to 1:6,400. IgM and IgG antibodies

specific to murine typhus antigens were detected by the addition of FITC-labelled anti-human IgM and IgG antibodies, respectively. Antibody binding was determined using a fluorescent microscope (Olympus BX50; Olympus Corporation, Japan). Known positive and negative control sera were run with each experiment.

2.3.1.1.3.2 Serological tests for other rickettsial infections

These were performed at Unité des Rickettsies, Faculté de Médecine, Université de la Méditerranée, Marseille, France (Professor Didier Raoult). The microimmunofluorescent antibody test was performed using a pooled panel antigen of *Coxiella burnettii* (Q fever), *Rickettsial honei* (Flinders Island spotted fever), *R. helvetica* (spotted fever group), *R. japonica* (Japanese spotted fever), *R. felis* (cat-flea typhus), *Bartonella henselae*, *B. quintana*, and *Anaplasma phagocytophila*, *Ehrlichia chaffeensis*, as described previously.[215] In brief, pooled rickettsial antigens were prepared from inactivated, purified, yolk-sac culture to 200µg of rickettsiae/mL and spotted onto microscopic glass slides. Serial 2-fold serum dilutions using phosphate-buffered saline containing 3% non-fat powdered milk to reduce non-specific background fluorescence were added to slides and incubated in a moist chamber for 30 min at 37°C.[158] After washing and drying, the binding sera were detected by using FITC-labelled goat anti-human IgM and IgG antibodies. Dried slides were mounted with buffered glycerol (pH 8.0) and examined with a fluorescence microscope at 400×. Known positive and negative control sera were run with each experiment.

2.3.1.2 Conventional blood culture and culture for *Leptospira* spp.

2.3.1.2.1 Haemoculture for aerobic bacteria

Ten-millilitre of blood taken aseptically from direct venepuncture was inoculated into 30 mL peptone-enriched tryptic soy broth, supplemented with brain-

heart infusion (BHI) and activated charcoal (BacT/ALERT® FA; bio Mérieux INDUSTRY, USA), and incubated aerobically at 37°C, in an automated incubator, for a maximum of 7 days for aerobic bacterial culture. These were sub-cultured onto horse-blood agar at 24, 48, and day 7 or at intervening time points if the medium became cloudy.

2.3.1.2.2 Haemoculture and CSF culture for Leptospira spp.

A 10-mL blood sample was collected into a sterile tube containing 250 U of heparin sodium (Heparin Leo; Leo Pharma, UK) from all patients. CSF was taken if clinically indicated and collected into a sterile bottle. Culture of *Leptospira* spp. was performed by adding 100μL heparin whole blood or 200μL plasma into individual 5-ml sterile tube plastic flat-based screw-cap tubes (Sterilin; Barloworld Scientific Ltd., UK) containing 3 ml of Ellinghausen-McCullough-Johnson-Harris (EMJH) media supplemented with 3% rabbit serum and 0.1% agarose. These were incubated aerobically and protected from light at room temperature (25 to 30°C) for at least six months.[319] The cultures were examined weekly for three months and then every 2 to 4 weeks for a further 3 months by placing one drop of the culture onto a microscopic glass slide and examining with dark-field microscopy at ×200 magnification. Positive cultures were sent to the WHO/FAO/OIE Collaborating Centre for Reference and Research on Leptospirosis, Australia, for identification using the cross-agglutinin absorption test (CAAT).[1]

2.3.2 Baseline investigations

Complete blood counts, including haemoglobin level, haematocrit, white blood, differential cell and platelet count were performed on 1mL EDTA blood on admission. Serum from 10mL clotted blood was collected on admission for tests of renal function

(blood urea nitrogen (BUN) and creatinine levels), liver function (total and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), albumin, globulin, and cholesterol), electrolytes (sodium, potassium, chloride, and bicarbonate), and muscle enzymes (creatine kinase (CK), creatine kinase MB (mass), and lactate dehydrogenase). A further 4.5mL citrated blood was collected for the coagulation assays described in detail in **Chapter IV**.

2.3.3 Pulmonary manifestations and chest radiographs

2.3.3.1 Patients

Patients enrolled into the acute febrile illness study in Udon Thani Regional Hospital between October 2000 and December 2002 who were diagnosed as having either leptospirosis and/or scrub typhus according to the criteria described above and whose chest radiographs were available for review were eligible for study.

2.3.3.2 Review of chest radiographs

Each chest radiograph underwent initial assessment to determine whether it was of sufficient quality and appropriate exposure. Acceptable chest radiographs were then simultaneously reviewed by a chest physician (Ass Prof Nitipatana Chierakul), and a radiologist (Ass Prof Orasa Chawalparit), both of whom are from the Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, who were blinded to clinical information. A report was reached after discussion and consensus.

2.3.3.3 Vascular pedicle width and cardiothoracic ratio

Pulmonary involvement was defined as of the presence of an abnormal chest radiograph involving lung parenchyma with or without concomitant respiratory symptoms.

The vascular pedicle width (VPW) was measured as described by Milne,[185] by dropping a perpendicular line from the point at which the left subclavian artery exits the aortic arch, and measuring across to the point at which the superior vena cava crosses the right mainstem bronchus as shown in **Figure 2.1**.[88] The cardiothoracic (CT) ratio was calculated by dividing the widest transverse diameter of the cardiac silhouette by the widest transverse diameter of the thorax above the diaphragm. Cardiomegaly was defined as a CT ratio greater than 0.5 in an upright position or 0.55 if in a supine position.

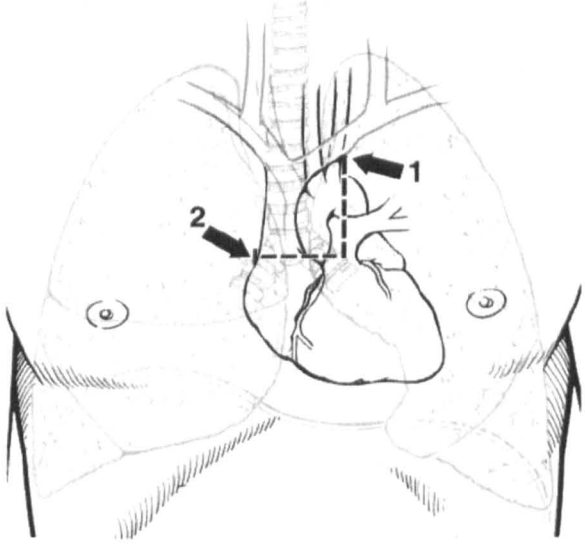

Figure 2.1 The measurement of vascular pedicle width

Patients with VPW of greater than 53 or 70mm in upright or supine position, respectively, coupled with a CT ratio of >0.55 are more than three times more likely to have pulmonary artery occlusion pressure >18mmHg than are patients without these findings. The increased VPW together with diffuse pulmonary infiltrates and cardiomegaly reflects hydrostatic pulmonary oedema, whereas a value lesser than these reflect permeability pulmonary oedema.[88] The test had 74% sensitivity and 94% specificity in a prospective evaluation.[120]

2.4 Diagnosis

2.4.1 Diagnosis of leptospirosis

Leptospirosis was diagnosed if *Leptospira* were isolated from blood and/or CSF, and/or if MAT demonstrated a 4-fold or greater rise in MAT titre between acute and convalescent sera to at least 1:200, or a single titre equal to or greater than 1:400.[4]

2.4.2 Diagnosis of scrub typhus

A positive serological result for scrub typhus infection was defined as a single IFA IgM titre against *O. tsutsugamushi* of equal to or greater than 1:400 or a 4-fold or greater rise in IFA IgM titre to at least 1:200, or a single IgG titre equal to or greater than 1:800 or a 4-fold or greater rise in IFA IgG titre to at least 1:200. The sensitivity of this cut-off is 48-54% with 96-98% specificity.[38]

2.4.3 Diagnosis of murine typhus

A positive serological result for murine typhus infection was defined as a single IFA IgM titre against *R. typhi* equal to or greater than 1:400 or a 4-fold or greater rise in IFA IgM titre to at least 1:200.[38]

2.4.4 Diagnosis of Q fever

Coxiella burnettii demonstrates antigenic phase variation termed virulent phase I and avirulent phase II. A positive serological result for acute Q fever was defined as a combination of a single cut-off titre of phase II IgM and IgG against *C. burnettii* that was equal to or greater than 1: 50 and equal to or greater than 1:200 , respectively, or a 4-fold or greater rise in IgM and/or IgG titre.[82]

2.5 *Database and statistical analysis*

Clinical information was recorded into the computer programme FileMaker Pro version 6.1 (FileMaker, Inc., California, USA). Detailed analyses were undertaken to compare demographic data, clinical history and examination, clinical course and outcome, and results of investigations in patients with leptospirosis and scrub typhus. Data were given as medians and ranges. Continuous variables were compared between leptospirosis and scrub typhus patients using the student t-test or Mann-Whitney U test, as appropriate. Categorical variables were compared between the two groups using the $\chi 2$ test or Fisher's exact test, as appropriate. Backward, stepwise, multiple logistic regression models were applied to determine the independent associations for fatality and the presence of pulmonary involvements for each disease. All variables which were associated biologically with death or the presence of pulmonary involvement in the initial model were included in these final logistic regression models. All analyses were performed using the statistical computing package STATA/SE, version 9.0 for Windows (Stata Corporation, Texas, USA).

2.6 *Summary diagram of study*

The summary flowchart of patients recruited into each part of study is shown in **Figure 2.2**.

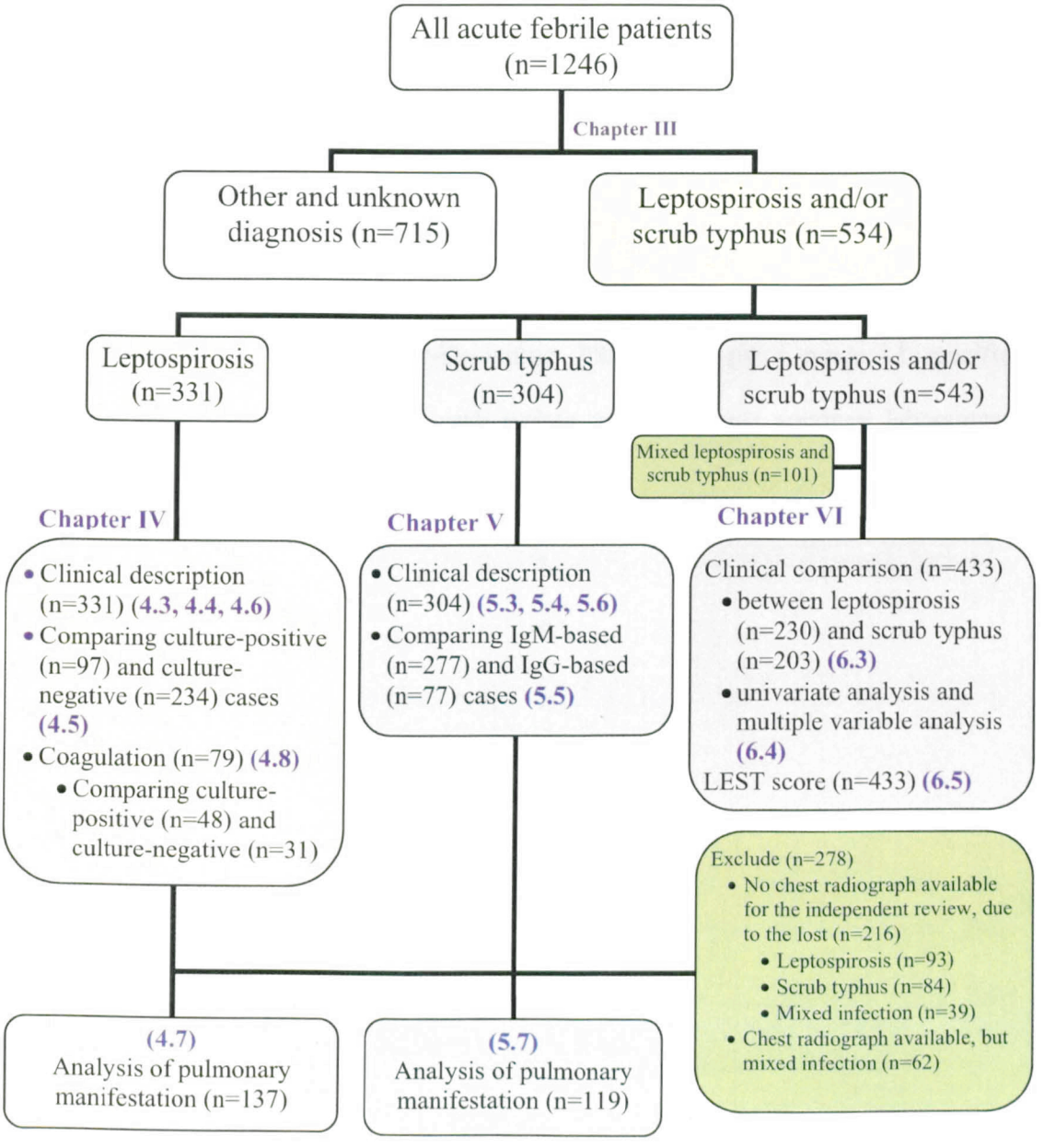

Figure 2.2 Flow chart illustrating patients included in each stage of study

Purple figures in brackets represent the chapter sections in this thesis.

Chapter III

Result I: Epidemiology of acute febrile illness

3.1 Chapter contents

Acute febrile illness (AFI) is common in Udon Thani, northeast Thailand. Diagnosis of the causative diseases is mostly based on clinical manifestations in combination with the results of routine laboratory (haematology and biochemistry) and radiological tests. The aim of the study was to describe the epidemiology of AFI in Udon Thani during October 2000-December 2002 based on a range of specific diagnostic tests. Leptospirosis and scrub typhus were the most common laboratory-confirmed diagnoses, and are described in detail.

3.2 Acute febrile illness in Udon Thani

3.2.1 General information

Udon Thani, a province in the northeast of Thailand covering an area of 11,730 km^2, is located 564 km from Bangkok to the northeast, on a plateau that is approximately 187 meters above sea level. The province borders Nong Khai to the north, Khon Kaen to the south, Sakon Nakhon to the east and Nongbualamphu and Loei to the west. Most of the land area is predominantly covered with rice fields, with some regions of forests and hills. Udon Thani is administratively divided into 20 districts with each containing a local, district hospital. There are three seasons in the year, summer, rainy and winter. The hottest month is April and the coldest month is January. The population of the province is approximately 1.4 million, 70% of the whole population are agricultural workers or family, including rice farming, corn farming, pig and cattle farming. The average income is around 120 THB (3.4 USD)/person/day. Udon Thani Regional Hospital at Muang District, the centre of the province, is the referral hospital for other district hospitals.

3.2.2 Diagnosis

A total of 1,249 patients with AFI were enrolled into the study between October 2000 and December 2002. The diagnoses of these patients are shown in **Table 3.1**. Leptospirosis was the most common diagnosis (n=331 [26.5%]) followed by scrub typhus (n=304 [24.3%]).

Table 3.1 Diagnosis of 1,249 patients with acute febrile illness admitted to Udon Thani Regional Hospital during October 2000-December 2002

Diagnosis	Number of cases (%)
Leptospirosis	215 (17.2)
Leptospirosis and scrub typhus	101 (8.1)
Leptospirosis and other infections	15 (1.2)
Scrub typhus	189 (15.1)
Scrub typhus and other infections	14 (1.1)
Murine typhus	23 (1.8)
Q fever	2 (0.2)
Bacterial septicaemia§ (other than *B. pseudomallei*)	40 (3.2)
Melioidosis (*B. pseudomallei*)	5 (0.4)
Malaria	3 (0.2)
Dengue fever/dengue haemorrhagic fever	39 (3.1)
Acute flavivirus infection (including Japanese Encephalitis infection)	5 (0.4)
Aseptic meningitis, cause unknown	2 (0.2)
Eosinophilic meningitis, cause unknown	3 (0.2)
HIV infection, newly diagnosed	7 (0.6)
Other diagnoses	21 (1.7)
Unknown	565 (45.2)

§They were *Escherichia coli* (15), *Klebseilla pneumoniae* (5), *Pseudomonas aeruginosa* (5), *Staphylococcus aureus* (4), *Acinetobacter baumannii* (2) and one each of *A. lwoffi*, *Enterococcus faecalis*, *Enterococcus* spp., *Acinetobacter* spp., *Corynbacterium jeikeium*, *K. oxytoca*, Gr. D *Salmonella*, *Streptococcus pneumoniae* and *Streptococcus* spp.

Of those patients with leptospirosis and scrub typhus, 101 (8.1%) patients had concomitant leptospirosis and scrub typhus infection as defined by the criteria for diagnosis in **Chapter II**. Sixty seven (66.3%) patients had a diagnosis of leptospirosis based on either culture-positive or a four-fold or greater rising MAT titre and had a diagnosis of scrub typhus based on a four-fold or greater rising of IFA titres, which are considered to be the confirmative diagnostic titres for these diseases (**Table 3.2**).

The 230 (18.4%) patients with leptospirosis without scrub typhus infection were diagnosed by culture in 85 (37.0%) patients, a four-fold or greater rise in MAT titre in 121 (52.6%) patients, and a single MAT titre of equal to or greater than 1:400 in 24

(10.4%) patients (1:400 [n=5], 1:800 [n=8], 1:1,600 [n=6], 1:3,200 [n=4], and 1:6,400 [n=1]).

Table 3.2 Basis for diagnosis of 101 patients with concomitant leptospirosis and scrub typhus infections

Scrub typhus diagnosis	Leptospirosis diagnosis		
	Culture-confirmed (n=12)	Four-fold or greater rise of MAT titre (n=71)	MAT titre ≥1:400§(n=18)
Four-fold or greater rise of IFA IgM (n=64)	6	52	6
Four-fold or greater rise of IFA IgG (n=11)	2	7	2
IFA IgM ≥1:400‡ (n=15)	0	7	8
IFA IgG ≥1:800† (n=11)	4	5	2

§MAT titre 1:400 (n=9), 1:800 (n=4), 1:1,600 (n=1), 1:3,200 (n=3), and >1:6,400 (n=1)

‡IgM titre 1:400 (n=1), 1:800 (n=4), 1:1,600 (n=2), 1:3.200 (n=1), and ≥1:6,400 (n=7)

†IgG titre 1:800 (n=6), 1:1,600 (n=2), 1:3,200 (n=1), and ≥6,400 (n=2)

There were 203 (16.3%) patients diagnosed as having scrub typhus without leptospirosis infection. Ninety-eight (48.3%) of these were diagnosed by a four-fold or greater rise in either IFA IgM (n=67) or IFA IgG (n=31), by a single IFA IgM titre of equal to or greater than 1:400 in 81 (39.9%) patients (1:400 [n=4], 1:800 [n=5], 1:1,600 [n=14], 1:3,200 [n=13], and ≥1:6,400 [n=45]), or by IFA IgG titre equal to or greater than 1:800 in 24 (11.8%) patients (1:800 [n=8], 1:1,600 [n=7], 1:3200 [n=6], and ≥1:6,400 [n=3]).

There were 395 (31.6%) patients of whom only admission sera could be obtained, since patients did not come back for follow up. This is a common and important problem for the epidemiological study in the difficult to diagnose, but easy to treat or self-limited diseases in which the diagnosis is based on the serology. Haemoculture was successfully performed in every patient. The causes of acute febrile illness were unknown in 565 (45.2%) patients despite receiving the diagnostic

laboratory tests described in **Chapter II**. Of these, 255 (45.1%) patients had a single serum collected during hospitalisation, but were then lost to follow up or died so a convalescent serum sample could not be obtained.

3.2.3 Seasonal variation

The number of patients admitted per month is shown in **Figure 3.1**. Most patients were admitted during the rainy season months (between June and October). The incidence of leptospirosis peaked in September, while that of scrub typhus peaked in October.

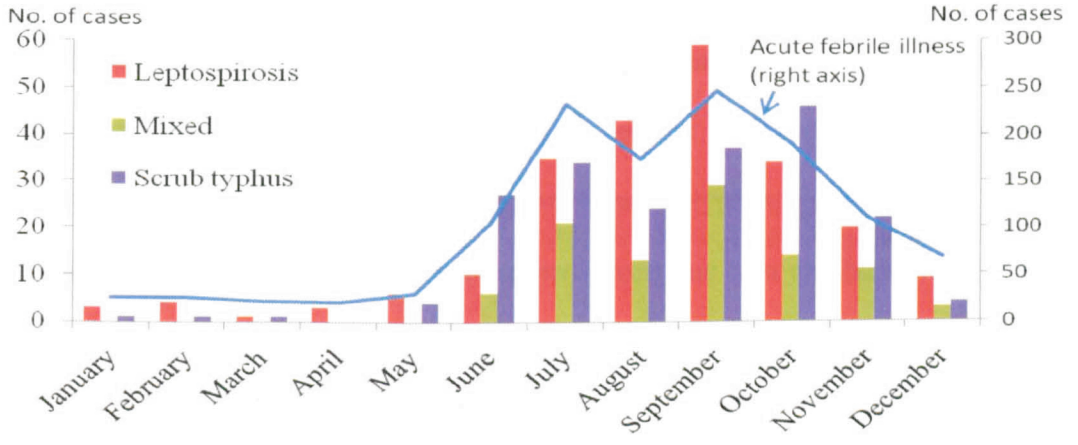

Figure 3.1 Total patients recruited by month with acute febrile illness, leptospirosis or scrub typhus

Right and left Y-axis are represent the different scale, bar graphs of leptospirosis, scrub typhus and mixed infections being read using the left axis, and the number of total acute febrile patients (blue line) being read using the right axis.

3.2.4 Risk factors and history of exposure

The history of exposure to natural water sources or animals is shown in **Table 3.3**. The majority of the types of exposure are considered to be associated with the risk of developing leptospirosis.

3.2.5 Demographic data and outcomes of acute febrile illness patients

The median (interquartile range [IQR]) age of patients enrolled into the study was 37 (26-51) years. There were 846 (67.7%) males with a male to female ratio of 2:1. The majority of patients (68%) were rice farmers or lived in a rice farming family. The median (IQR) duration of fever before admission to the hospital was 4 (3-6) days.

The overall mortality was 6.4%. A total of 80 deaths occurred in the study group, of which 31 (38.8%) died within 48 hours of admission. There were 30 patients for whom the diagnoses were unknown who died from pulmonary haemorrhage, with the presence of jaundice, renal failure, shock, anaemia and thrombocytopenia. All but one of these patients had only a single serum specimen taken prior to death. Blood culture from these patients was negative for aerobic bacteria and *Leptospira* and serological tests were negative for leptospirosis, scrub typhus, murine typhus, dengue and other arboviruses. No autopsies were performed due to a combination of limited facilities and cultural beliefs.

Table 3.3 Risk factors, modes and duration of exposure among 534 patients with leptospirosis and/or scrub typhus

	Number of cases (%)				*P*-value§
	Non-scrub typhus, Non-leptospirosis (n=895)	Mixed infection (n=101)	Leptospirosis (n=230)	Scrub typhus (n=203)	
Occupation: Rice farmer	403 (56.4)	79 (78.2)	179 (77.8)	168 (82.8)	0.20
Overall exposure	419 (62.5)	91 (93.8)	217 (94.8)	175 (87.1)	0.005
Farming (rice and/or non-rice)	177 (26.9)	46 (47.4)	120 (53.3)	93 (46.3)	0.15
Fishing	195 (29.6)	45 (46.4)	100 (43.7)	71 (35.3)	0.08
Swimming in natural water sources	41 (6.3)	0	7 (3.1)	7 (3.5)	0.83
Walking in flood	35 (5.4)	7 (7.2)	22 (9.8)	15 (7.5)	0.40
Killing or contact with animals	11 (1.7)	3 (3.1)	5 (2.2)	9 (4.5)	0.19
Duration of the above exposure					0.59
less than 1 hour		7 (7.7)	17 (7.8)	19 (10.9)	
1-2 hours		15 (16.5)	43 (19.8)	30 (17.1)	
>2-4 hours		19 (20.9)	42 (19.4)	28 (16.0)	
>4-6 hours		12 (13.2)	28 (12.0)	35 (20.0)	
>6-8 hours		13 (14.3)	23 (10.6)	22 (12.6)	
more than 8 hours		25 (27.5)	64 (29.5)	41 (23.4)	
Presence of wound or penetrating injury	216 (33.7)	49 (50.5)	130 (57.3)	59 (29.5)	<0.001

§Two groups comparison; leptospirosis and scrub typhus

3.3 Discussion

The study was designed to determine the causes of AFI in consecutive patients presenting to a hospital in northeast Thailand over a two years. Leptospirosis and scrub typhus were the two most common diseases found in this study. Other rickettsial diseases apart from scrub typhus and murine typhus have rarely been reported in Thailand. Q fever, a rickettsial disease caused by *C. burnettii* was diagnosed in two patients. Detailed clinical data of these two patients together with seven more Thai patients with Q fever have been reported separately.[260]

Leptospirosis was the most common diagnosis in this study. A previous multi-centre study conducted in the same region between 1991-1993 found that scrub typhus was the most common cause of acute undifferentiated fever rather than leptospirosis.[167] One explanation for this difference is that there was an extended outbreak of leptospirosis in the northeast between 1997 and 2002.

The second most common diagnosis in this study was scrub typhus, which accounted for nearly 25% of patients. Although scrub typhus was the most common cause of AFI during 1991-1993,[167] it accounted for only 7.5% of cases in that study. Comparing the results of the study described here with national data from the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, suggests that cases of scrub typhus are severely under-reported. During the period of the study, 2000-2002, the number of scrub typhus cases recorded by MOPH for the entire Udon Thani province was 0, 10, and 10 cases for each year, respectively. A comparison of the number of reported and true cases of scrub typhus in Udon Thani province is shown in **Figure 3.2**. Under-reporting may relate to the lack of diagnostic tests, unusual presentations of disease and lack of awareness of the disease.

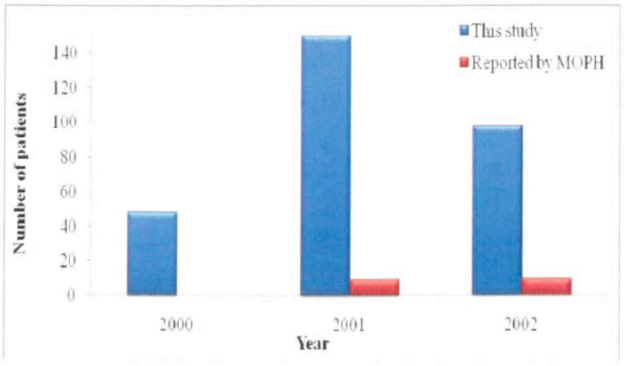

Figure 3.2 Number of patients with scrub typhus in the study diagnosed by year compared with those reported in Udon Thani Province, by Bureau of Epidemiology, DDC, MOPH

Despite the diagnostic tests used in this study, the diagnoses for 45% of patients remain unknown. Nearly half of patients who had an unknown diagnosis had only a single admission serum specimen taken, the convalescent sera not being obtained either because of loss to follow up or because the patient died. However, the majority of patients with an unknown diagnosis recovered after a course of antibiotics, the most common used being either a third generation cephalosporin and/or doxycycline. The possible causes of fever in these patients include viral infections, other self-limiting infections, or infections which response to the antibiotics given, including scrub typhus, other rickettsioses, and leptospirosis.

Diagnosing infectious diseases on the basis of serological tests represents a major problem, since detectable antibody may take several weeks to develop and admission antibody is almost always undetectable, especially if the patient seeks rapid medical attention. The estimated cost of investigations per patient in this study was more than 5,000 Baht, whereas the minimum wage for a worker is currently 203 Baht/day. Rice farmers often receive a minimum wage. Diagnostic tests that are available in Udon Thani hospital and provided free include blood culture, routine biochemical and haematological laboratory tests, insensitive serological tests for leptospirosis (MCAT) and the Weil Felix test for scrub typhus. All other serological

tests such as IFA, MAT, dengue ELISA, and other Flavivirus serological tests including Japanese B encephalitis are unavailable in the hospital, and patients are expected to pay for these. The usual outcome is that such tests are not ordered, and if the diagnosis cannot be reached using the battery of free tests the patient is treated based on clinical grounds alone.

The overall mortality in this study was around 6.5%, which was higher than the mortality for patients with a confirmed diagnosis of leptospirosis or scrub typhus. Most deaths occurred in patients who did not have a diagnosis, the majority of whom had a clinical syndrome consistent with severe leptospirosis (Weil's disease) and death associated with pulmonary haemorrhage. Leptospirosis or scrub typhus could not be diagnosed because of the lack of a second serum sample. Autopsy is not performed in northeast Thailand, and defining the cause of death in such cases in the future will depend on the use of sensitive molecular tests that can be used on the day of admission. Unfortunately these cases were excluded in the detailed comparison of leptospirosis and scrub typhus in subsequent chapters. However, the specimens from these patients can be evaluated with more advanced and sensitive molecular methods in the future, and re-analysis after inclusion of these fatal cases can be accomplished.

The incidence of both leptospirosis and scrub typhus showed marked seasonal variation. Leptospirosis in this study peaked in September, a month later than the national figures shown in **Figure 1.6**. Two peaks were observed for scrub typhus, with a smaller peak being observed in July and a larger peak in October. This contrasts with national figures, which also describe two peaks but with the highest peak observed during June and July and a smaller peak in October (**Figure 1.10**). It is not clear whether this represents bias in reporting during certain months of the calendar year (perhaps due to changes in junior medical staff), or because of some other factor such as

differences in timing of disease over the northeast as a whole compared with that in Ubon Thani.

Overall 67% of the study population was farmers. Activities undertaken by farmers include planting and harvest of rice and other crops, pig farming and raising of other animals and fishing. The overall water exposure history was significantly higher in patients with leptospirosis and/or scrub typhus groups compared with other patients, and was also higher in patients with leptospirosis than patients with scrub typhus. Patients with leptospirosis also reported more cut wounds than patients with scrub typhus. This suggests that the exposure history remains useful, but may be more relevant in areas where few people are exposed on a regular basis to potentially contaminated water sources. The higher number of patients with scrub typhus reported from the north of the country[5] may be explained in part by geographical differences, with less water and more forestry in the north and occupational activities associate with work in or near forested areas that form the natural habitat for the vectors of scrub typhus.

Concomitant leptospirosis and scrub typhus infections occurred in 8% of patients overall in this study. There are several possible reasons for this finding. Both infections were very common alone, and so disease prevalence is high for both conditions and working as a farmer is highly likely to predispose these individuals to both infections. Given this, it is plausible that both infections could occur together. This would be consistent with patients who had four-fold rising titre against both *O. tsutsugamushi* and *Leptospira* spp. A second explanation is that one or both tests show cross-reactivity, a possibility that could be evaluated using molecular tools such as PCR to detect bacterial DNA of either species in admission blood. Such assays have been described and warrant application on patient samples obtained during this study. A third explanation is that the cause of fever in this group was either scrub typhus or

leptospirosis, and that the alternate infection had occurred in the recent past. This is of particular relevance to patients who had a rising titre to one pathogen but a single or sustained high titre to the other. Further studies are required to determine the relative importance of each of these possibilities.

3.4 Chapter summary

Leptospirosis and scrub typhus are the two most common causes of acute febrile illness in northeast Thailand and taken together account for nearly 45% of cases. Co-infections between the two were also common, with 30% of patients diagnosed with either scrub typhus or leptospirosis having both infections. Seasonal variation associated with the rainy season was shown to occur for both diseases. The diagnosis was unknown in half of the study group. This is likely to relate to the observation that death occurred in a proportion of patients before convalescent serology samples could be obtained, and because the spectrum of laboratory tests was limited. For example, testing was not performed for some viral infections including influenza and parainfluenza. Leptospirosis and scrub typhus are under-reported in the national figures, and this is likely to contribute towards a lack of awareness of these conditions among admitting physicians and healthcare policy makers. Pulmonary haemorrhage and multiorgan dysfunction were the most common features associated with study patients who died, a feature most consistent with a diagnosis of leptospirosis. This implies that empiric treatment of patients admitted with fever and severe clinical manifestations should receive antimicrobial drugs that are at least effective against *Leptospira* spp.

Chapter IV

Result II: Leptospirosis

4.1 Chapter contents

Leptospirosis is the most common cause of acute febrile illness in Northeast Thailand. Clinical manifestations are broad, and range from a mild, flu-like illness to fatal multiorgan involvement and death. Bleeding complications commonly occur in leptospirosis and one of the major causes of death is pulmonary haemorrhage. Coagulopathy and thrombocytopenia have been noted previously in association with severe leptospirosis, but their relationships with clinical bleeding and fatal outcomes have not been described. The aims of the study were to:

1. Describe in detail the clinical manifestations, laboratory findings, and outcomes of patients with leptospirosis.
2. Compare the clinical manifestations, laboratory findings and outcomes between patients with leptospirosis who were diagnosed by culture of *Leptospira* spp. versus patients who were diagnosed based on the MAT alone.
3. Determine the incidence of pulmonary involvement and describe the pulmonary manifestations and chest radiographs findings in patients with pulmonary leptospirosis.
4. Determine the risk factors for severe pulmonary complications in patients with leptospirosis.
5. Describe the prevalence of coagulopathy and bleeding diathesis in patients with leptospirosis and the associated clinical features.

4.2 Patients and methods

4.2.1 Patients

4.2.1.1 Clinical and laboratory manifestations

A total of 331 patients who were diagnosed as having leptospirosis in the acute febrile illness cohort were recruited in the analysis, regardless of other concomitant infections.

4.2.1.2 Pulmonary manifestations of leptospirosis

Patients whose chest radiographs and clinical details were available for the review were eligible for the detailed analysis of pulmonary manifestations.

4.2.1.3 Coagulopathy and bleeding diathesis in leptospirosis

As part of the acute febrile illness study, consecutive patients with confirmed leptospirosis were enrolled into the coagulation study. Blood samples obtained from 33 healthy Thai donors who were not receiving any medication were used as controls.

4.2.2 Methods

Data collection and analysis was performed as described in **Chapter II**. The coagulation study was performed as described below.

4.2.2.1 Coagulation tests

4.2.2.1.1 Blood collections

4.5 mL of blood was collected into 3.2% sodium citrate tubes (BD Vacutainer® Blood Collection Tube; Becton, Dickinson and Company, New Jersey, USA). Plasma was separated into two tubes, one of which was used for immediate coagulation tests

and the second was stored immediately at -70°C until further assays could be performed.

4.2.2.1.2 Assays

Prothrombin time (PT) and activated partial thromboplastin time (APTT) were performed immediately on citrated plasma at the Udon Thani hospital laboratory using Thromborel® S and Dade® Actin® FS Activated PTT Reagents (Dade Behring, Marburg, Germany), respectively.

Stored citrated plasma was transferred to the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University on dry ice for further testing. Fibrinogen, F1+2, and TAT were measured by specific enzyme-linked immunosorbent assays (ELISA), according to the manufacturer's instructions [fibrinogen: PT-derived fibrinogen method using Thromborel® S as the PT reagent on Behring Coagulation Timer® , F1+2: Enzygnost® F1+2 micro, and TAT: Enzygnost® TAT micro (Dade Behring, Marburg, Germany)]. D-dimer was measured using a latex-enhanced, turbidimetric test, D-Dimer Plus (Dade Behring, Marburg, Germany). Monoclonal antibodies specific for each assay were used in all of the test systems.

4.2.3 Clinical assessment

4.2.3.1 Clinical severity

Patients were defined as having severe leptospirosis if ≥1 of the following were present: renal involvement (oliguria or a creatinine level >2.5mg/dL), jaundice (total bilirubin level >2.5mg/dL), clinical bleeding abnormalities, hypotension (systolic blood pressure <90mmHg or diastolic blood pressure <60mmHg), or the presence of respiratory distress or respiratory failure.[259]

Clinical severity was assessed using the Acute Physiology and Chronic Health Evaluation (APACHE) II score[151] and Sepsis-Related Organ Failure Assessment (SOFA) score.[294]

4.2.3.2 Disseminated intravascular coagulation (DIC) score

A 'DIC score' was calculated for each patient using the platelet count and the D-dimer, prothrombin time, and fibrinogen levels according to the algorithm for the diagnosis of overt DIC recommended by the DIC scientific subcommittee (SSC) of the International Society for Thrombosis and Haemostasis (ISTH).[269] This DIC score was calculated by assigning 1 point for each of the following: a) a platelet count of <100 but >50 $\times 10^9$/mL; b) a prolonged PT of >3 sec but <6 sec; c) a fibrinogen <1.0 g/L. A platelet count <50 $\times 10^9$/mL, a prolonged PT of ≥6 sec, and an elevated D-dimer ≥2 times but <5 times the upper limit of normal were each assigned 2 points, and a D-dimer of ≥5 times the upper limit of normal scored 3 points. A score equal to or more than 5 was compatible with overt-DIC, whereas a score of less than 5 may be indicative (but is not affirmative) of non-overt DIC. The algorithm for non-overt DIC was not applied since protein C and antithrombin were not measured. No follow up coagulation tests were performed in this study.

4.2.4 Statistical analysis

Statistical analyses were performed as described in **Chapter II**. For the coagulation study, the nonparametric Spearman correlation (ρ) was used to demonstrate correlations between laboratory test results and APACHE II and SOFA scores. All analyses in the coagulation studies were performed using the statistical computing package STATA/SE, version 9.0 for Windows (StataCorp, Texas, USA), and the

graphics were constructed using GraphPad Prism, version 4 for Windows (GraphPad Software, Inc., CA, USA).

Survival rates were plotted by the Kaplan-Meier method. Events were defined as in-hospital deaths. Time-to-event was compared between groups of interest using the logrank test or Wilcoxon test as appropriate.

Univariate logistic regression analyses were performed using variables that possibly predicted a fatal outcome based on a literature review. These included age, sex, the presence of *Leptospira* in blood or CSF, the presence of oliguria, shock, haemoptysis, diarrhoea, anaemia, abnormal bleeding including gastrointestinal bleeding, pulmonary haemorrhage, thrombocytopenia, jaundice, elevation of liver enzymes, acidosis, hypo- or hyperkalaemia, hypoalbuminaemia, and renal failure. Multiple-variable logistic regression models were applied to determine the independent associations for a fatal outcome. All variables which were associated biologically with death in the univariate analysis were included in these final logistic regression models. The same analysis methods were used to define the risk factors of having severe pulmonary haemorrhage outcome.

4.3 Epidemiology of leptospirosis

4.3.1 Diagnosis

Leptospirosis was diagnosed in 331 (26.5%) patients based on culture of *Leptospira* spp. and/or serological tests. Of these, 97 (29.3%) patients were culture-positive for *Leptospira* from either blood and/or cerebrospinal fluid. The remaining 234 (70.7%) patients were diagnosed on the basis of a four-fold rise in MAT titre to at least 1:200 (n=193) or a single MAT titre of $\geq$ 1:400 in patients in whom only a single serum

was obtained (n=41). Forty-six (48.9%) patients in the culture-confirmed group had negative serological tests for leptospirosis.

Of those 331 patients with leptospirosis, 117 (35.3%) had concomitant infection including scrub typhus (n=101, 30.5%) or bacterial septicaemia (n=10, 3.0%). Details of concomitant diagnoses are shown in **Table 4.1**.

Table 4.1 Diagnosis of 331 patients with leptospirosis

Diagnosis	Number of cases (%)
Leptospirosis only	214 (64.7)
Leptospirosis combined with another concurrent infection	111 (33.5)
Scrub typhus	96 (29.0)
Murine typhus	4 (1.2)
Bacterial septicaemia	8 (2.4)
Escherichia coli	1 (0.3)
Acinetobacter baumannii	1 (0.3)
Klebseilla pneumoniae	1 (0.3)
Salmonella Group D	1 (0.3)
Staphylococcus aureus	3 (0.9)
Enterococcus spp.	1 (0.3)
K. pneumoniae pneumonia	2 (0.6)
E. coli acute pyelonephritis	1 (0.3)
Leptospirosis combined with other two concurrent infections	6 (1.8)
Scrub typhus and murine typhus	4 (1.2)
Scrub typhus and *Klebseilla* spp. septicaemia	1 (0.3)
Murine typhus and *A. baumannii* septicaemia	1 (0.3)

4.3.2 *Leptospira* serovar determination

Of 97 patients who were culture-positive for *Leptospira* spp., the organism was isolated from both CSF and blood in one patient, from CSF alone in one patient, and from blood alone in the remainder. The serovars (sv.) determined for cultured *Leptospira* spp. were as follows: *L. interrogans* sv. Autumnalis (74), *L. interrogans* sv. Pyrogenes (4), *L. interrogans* sv. Medanensis (4), *L. borgpetersenii* sv. Javanica (3), *L. interrogans* sv. Bataviae (2), *L. interrogans* sv. Grippotyphosa (1), *L. interrogans* sv. Canicola (1), and unidentified serovars (8).

4.3.3 Risk factors and history of exposure

A total of 308 (94.5%) patients gave a history of contact with natural water sources (rivers, pond, lakes or paddy fields) or animals (cattle, pigs, dogs). The two most common modes of contact with water were occupational exposure (either rice and/or non-rice farming) and fishing (**Table 3.3** in **Chapter III**).

One hundred and ninety-two (58.0%) patients had a history of a wound or penetrating injury, within the past two weeks involving the foot (n=150), leg (n=53), hand (n=20), arm (n=7), or trunk (n=2). A total of 166 (50.2%) patients had an open wound that came into contact with natural water. There were 46 (14.2%) patients with a history of frequent alcohol consumption, but only six patients had a history of chronic liver disease.

4.4 Clinical manifestations and laboratory findings of leptospirosis

The male:female ratio was 3.2:1. The median (IQR) age was 37 (27-50) years, and median (IQR) duration of fever before admission was 4 (3-6) days.

4.4.1 Presenting symptoms and signs

Demographic data and clinical symptoms and signs are shown in **Table 4.2**. Fever, chills, headache and myalgia were the most prominent findings.

Table 4.2 Demographic data and clinical manifestations for 331 patients with leptospirosis

Parameters	Number of cases (%)			*P*-value‡
	All leptospirosis (n=331)	Culture +ve (n=97)	Culture-ve (n=234)	
Sex: male	253 (76.4)	75 (77.3)	178 (76.1)	0.81
Median (IQR) age, year	37 (27-50)	34 (24-47)	39 (29-50)	0.08§
Median (IQR) duration of symptoms, days	4 (3-6)	3 (2-4)	5 (4-6)	<0.001§
Median (IQR) temperature, °C	38.0 (37.1-39.0)	38.5 (37.7-39.2)	37.8 (36.9-38.7)	<0.001§
Median (IQR) highest temperature, °C	38.7 (38.0-39.6)	39.3 (38.7-39.8)	38.4 (37.9-39.3)	<0.001§
Median (IQR) MAP, mmHg	73 (60-83)	80 (67-87)	70 (60-83)	0.005§
Median (IQR) pulse rate, /min	94 (84-108)	94 (84-104)	94 (84-108)	0.55§
Median (IQR) respiratory rate, /min	24 (20-26)	24 (20-24)	24 (20-26)	0.39§
Tachypnoea (RR ≥24/min)	201 (61.7)	57 (58.8)	144 (62.9)	0.48
Fever	325 (99.7)	97 (100.0)	228 (99.6)	0.52
Chills	276 (84.7)	84 (86.6)	192 (83.8)	0.53
Myalgia	306 (93.9)	91 (93.8)	215 (93.9)	0.98
Muscle tenderness	77 (23.9)	25 (26.0)	52 (23.0)	0.56
Arthralgia	50 (15.3)	17 (17.5)	33 (14.4)	0.48
Wound	182 (56.5)	55 (57.3)	127 (56.2)	0.78
Skin rash	19 (5.8)	4 (4.1)	15 (6.6)	0.39
Subconjunctival haemorrhage	36 (11.2)	1 (1.1)	35 (15.4)	<0.001
Conjunctival suffusion	208 (62.8)	62 (63.9)	146 (62.4)	1.00
Palpitations	2 (0.6)	1 (1.0)	1 (0.4)	0.53
Chest pain	40 (12.3)	7 (7.2)	33 (14.4)	0.07
Tinnitus	12 (3.7)	2 (2.1)	10 (4.4)	0.31
Respiratory symptoms	170 (52.2)	44 (45.4)	126 (55.0)	0.11
Cough	119 (36.5)	29 (29.9)	90 (39.3)	0.11
Sputum	62 (19.0)	15 (15.5)	47 (20.5)	0.29
Dyspnoea	58 (17.8)	7 (7.2)	51 (22.3)	0.001
Sore throat	64 (19.6)	23 (23.7)	41 (17.9)	0.23
Jaundice	115 (35.5)	13 (13.7)	102 (44.5)	<0.001
Anorexia	217 (66.6)	59 (60.8)	158 (69.0)	0.15
Nausea	222 (68.1)	59 (60.8)	163 (71.2)	0.07
Vomiting	196 (60.1)	52 (53.6)	144 (62.9)	0.12
Diarrhoea	61 (18.7)	18 (18.6)	43 (18.8)	0.96
Abdominal pain	103 (31.6)	18 (18.6)	85 (37.1)	0.001
Headache	267 (81.9)	85 (87.6)	182 (79.5)	0.08
Confusion	27 (8.3)	2 (2.1)	25 (10.9)	0.01
Deterioration of consciousness	19 (5.9)	2 (2.1)	17 (7.4)	0.07
Convulsions	8 (2.5)	1 (1.03)	7 (3.1)	0.44
Stiff neck	31 (9.6)	10 (10.4)	21 (9.3)	0.75
Oliguria/anuria	72 (22.1)	10 (10.3)	62 (27.1)	0.001
Abnormal bleeding	106 (32.5)	14 (14.4)	92 (40.2)	<0.001
Epistaxis	29 (9.0)	4 (4.2)	25 (11.1)	0.05
Haemoptysis	36 (10.9)	7 (7.2)	29 (12.4)	0.17
Petechial haemorrhage	20 (6.2)	0	20 (8.9)	0.003
Haematemesis	12 (3.7)	2 (2.1)	10 (4.4)	0.52
Melaena	42 (12.9)	5 (5.2)	37 (16.2)	0.01
Haematuria	5 (1.5)	1 (1.0)	4 (1.8)	1.00

‡χ^2 test

§Mann-Whitney U test

4.4.1.1 Fever and associated symptoms

Almost all patients had a history of fever (99.7%), while the remainder were enrolled following the detection of a temperature of ≥37.8°C during hospitalisation without patient recognition. Despite the high percentage of myalgia (93.9%) reported by patients, muscle tenderness on physical examination was found in only 77 (23.9%) patients, almost all involving the calf muscles.

A distribution plot of fever and duration of symptoms is shown in **Figure 4.1**. The duration of symptoms was negatively correlated with the body temperature on admission ($|\rho| = 0.21$, $P < 0.001$). The analysis revealed a statistically significant trend in which the admission temperature was positively associated with the duration of illness (P-value for trend =0.002).

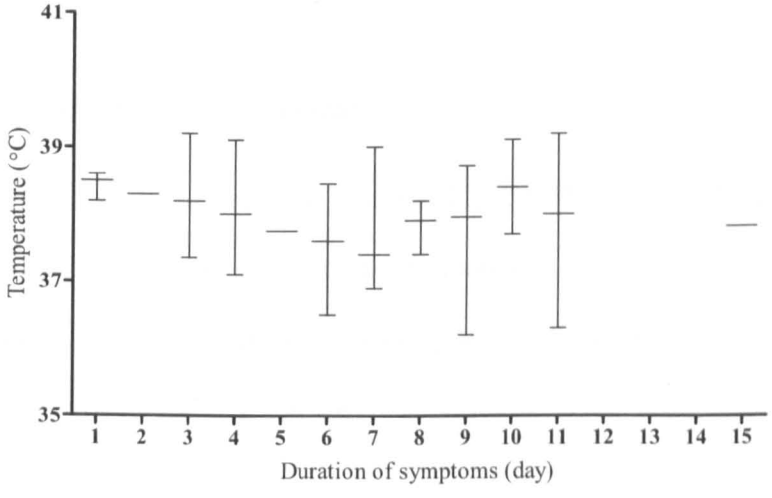

Figure 4.1 Median (range) of temperature on admission and duration of symptom

4.4.1.2 Headache and central nervous system

Headache occurred in 267 (81.9%) patients. Patients reported the severity of headache as mild, moderate or severe in 64 (24.0%), 130 (48.7%), and 73 (27.3%) cases, respectively. The onset of headache was insidious in 182 (68.2%) cases, whereas

sudden onset headache occurred in only 85 (31.8%) patients. This contrasts with textbooks descriptions that the main presenting symptom in most patients is sudden onset headache. The pattern of headaches were intermittent pain in 228 (85.4%) patients and persistent pain in 39 (14.6%) patients, and the character of headaches were dull aching pain and throbbing pain in 193 (77.3%) and 73 (27.3%), respectively.

The median (range) Glasgow Coma Score (GCS) was 15 (9-15). A stiff neck was found in 31 (9.6%) of patients, 4 of whom had no history of headache. Meningitis was found in 25 (7.8%) patients, all of whom had features of viral meningoencephalitis with a CSF cell count ranging from 0-295 cells/mL and monocytes predominating (median [IQR] %lymphocyte of 100 [93.5-100]). The CSF profiles from patients with leptospirosis are shown in **Appendix B**. CSF was clear in all patients. The median (IQR) protein and sugar concentrations were 42 (16.5-49.5)g/dL and 58 (54.5-63.5)mg/dL, respectively.

4.4.1.3 Hepatobiliary system

Icteric sclera or jaundice was found in 115 (35.5%) patients. Degrees of jaundice were judged by a single observer as mild, moderate, and severe in 50 (15.5%), 33 (10.3%), and 31 (9.6%), respectively. Seventy-one (21.5%) patients had hepatomegaly, and 15 (4.5%) patients had splenomegaly.

4.4.1.4 Renal system

Seventy-two (22.1%) patients presented with a history of oliguria (urine output less than150 mL within last 6 hours). Eight patients had a history of cloudy urine and 4 patients had dysuria. Flank pain occurred in 190 (58.3%) patients.

4.4.1.5 Haematological system and bleeding diathesis

One hundred and six (32.5%) patients had a history of abnormal bleeding. The most common site of bleeding was the gastrointestinal tract, including melaena (42 [12.9%]) and haematemesis (12 [3.7%]). Haemoptysis occurred in 36 (10.9%) of patients, but no massive haemoptysis (>150mL at a time or >600mL/day) was observed in the study patients. Petechial haemorrhage occurred in 20 (6.2%) patients who also had a platelet concentration of less than 100×10^9/mL, all except four of whom had a platelet concentration of less than 50×10^9/mL.

4.4.2 Laboratory findings

One hundred and ninety (58.3%) patients had thrombocytopenia (**Table 4.3**), 82 (25.2%) of whom had a platelet concentration less than 50×10^9/mL, and severe thrombocytopenia (platelet concentration less than 25×10^9/mL) was found in 34 (10.4%) patients.

One hundred and forty-nine (46.0%) patients had a normal serum creatinine (≤1mg/dL), including 7 patients who had a history of oliguria, 40 (12.3%) patients had serum creatinine between 1.0 and 1.5mg/dL, 59 (18.2%) patients had a serum creatinine of between 1.5mg/dL and 4.0mg/dL (39 (66.1%) patients with non-oliguria), and 76 (23.4%) patients had a serum creatinine level of ≥4.0mg/dL (34 (44.7%) patients with non-oliguria). Hypokalaemia on admission was found in 149 (46.1%) patients, 84 (56.4%) of which was in patients with impaired renal function.

The median (IQR) BUN:creatinine ratio was 12.5 (10-16). There were 200 (61.5%) patients with a BUN:creatinine ratio between 10 and 20, 39 (12.0%) patients had a ratio ≥20 which reflects a pre-renal cause of azotaemia, and the remainder had a ratio of less than 10.

Eight patients had an AST in excess of 300U/L, and 10 patients had an AP of more than four times the upper limit of the normal range, whereas 81(24.5%) patients had total bilirubin level greater than 5mg/dL.

Table 4.3 Laboratory findings for 331 patients with leptospirosis

Parameters	Median (IQR)			*P*-value§
	All leptospirosis (n=331)	Culture+ve (n=97)	Culture-ve (n=234)	
Sodium, mmol/L	139 (136-142)	140 (137-142)	139 (136-142)	0.10
Potassium, mmol/L	3.6 (3.3-4.0)	3.6 (3.3-3.9)	3.6 (3.3-4.0)	0.84
Bicarbonate, mmol/L	23 (21-26)	24.5 (23-27)	22 (20-25)	<0.001
Blood Urea Nitrogen, mg/dL	23 (14-46)	16 (12-23)	31 (15-71)	<0.001
Creatinine, mg/dL	1.5 (1.2-3.7)	1.3 (1.1-1.6)	2.2 (1.3-5.1)	<0.001
Total bilirubin, mg/dL	1.4 (0.8-4.1)	1.1 (0.6-1.8)	1.9 (0.8-7.9)	<0.001
Direct bilirubin, mg/dL	0.9 (0.4-2.8)	0.6 (0.4-1.0)	1.3 (0.5-5.3)	<0.001
AST, U/L	60 (40-111)	52 (38-84)	66 (41-120)	0.02
ALT, U/L	50 (31-74)	48 (28-75)	51 (33-74)	0.33
Alkaline phosphatase, U/L	120 (83-174)	98 (76-155)	125 (86-180)	0.01
Albumin, g/dL	3.5 (3.0-4.0)	3.9 (3.6-4.3)	3.2 (2.9-3.8)	<0.001
Globulin, g/dL	2.8 (2.4-3.1)	2.9 (2.5-3.1)	2.7 (2.4-3.1)	0.49
CPK, U/L	195 (90-482)	174 (86-354)	202 (91-543)	0.16
CK-MB mass, µg/mL	20.4 (15.4-31.7)	19.8 (14.6-27.3)	20.9 (15.7-31.7)	0.39
Lactate dehydrogenase, U/L	258 (208-377)	222 (179-302)	268 (213-399)	<0.001
Haemoglobin, g/dL	12.2 (10.6-13.7)	13.2 (12.1-14.3)	11.7 (9.9-13.1)	<0.001
Haematocrit, %	37 (32-41)	39 (37-42)	35 (31-39)	<0.001
Leukocyte, $\times 10^9$/L	10.7 (8.2-13.7)	10.2 (9.0-13.8)	10.9 (8.0-13.7)	0.73
% Neutrophil	84 (78-89)	85 (82-90)	83 (76-88)	<0.001
Platelets, $\times 10^9$/mL	122 (49-191)	162 (92-218)	96 (41-182)	<0.001

§Mann-Whitney U test

4.5 Culture-confirmed vs serological-confirmed leptospirosis

The clinical manifestations of 97 patients with culture-confirmed leptospirosis (culture-positive group) were compared with 234 patients with leptospirosis diagnosed by positive MAT only (culture-negative group).

There were no significant differences in median age, sex, occupational exposure, and risk factors between the two groups (**Table 4.2 and Table 4.3**). Patients in the culture-negative group had a significantly longer median (range) duration of symptoms prior to admission than those in the culture-positive group (5 (1-15) days and 3 (2-8) days, respectively, P=0.0001) (**Table 4.2**).

Most of the clinical signs and symptoms were similar in both culture-positive and culture-negative groups, with the exception that patients in the culture-negative group had a significantly lower body temperature (P<0.001), blood pressure (P=0.005) and significantly more patients with the presence of symptoms and signs associated with complications of severe disease. Detailed clinical manifestations comparing culture-positive and the culture-negative groups are shown in **Table 4.2**.

Liver function derangement was more common in the culture-negative group, in whom bilirubin levels (conjugated and total) were significantly higher compared with the culture-positive group (P<0.001, both). AST and AP levels were also significantly higher in the culture-negative group than the culture-positive group (P=0.02 and P=0.01, respectively), although the ALT level was not significant different between the two groups (P=0.33) (**Table 4.3**). All 10 patients who had AP level higher than 4 times of the upper limit of normal range were in the culture-negative group. The albumin level of the culture-negative group was also significantly lower than that of the culture-positive group (P<0.001) (**Table 4.3**).

The proportion of patients who had hypokalaemia was not different between the two groups on admission (42 (43.3%) *vs* 107 (46.9%), *P*=0.55), but rose in the culture-negative group during hospitalisation compared with the culture-positive group (55 (56.7%) *vs* 159 (69.7%), *P*=0.02) (**Table 4.4**).

Table 4.4 Complications of 331 patients with leptospirosis

Complications	Number of cases (%)			*P*-value‡
	All leptospirosis (n=331)	Culture +ve (n=97)	Culture-ve (n=234)	
On admission				
Shock	123 (38.1)	25 (26.0)	98 (43.2)	0.004
Anaemia	149 (45.0)	23 (23.7)	126 (53.9)	<0.001
Thrombocytopenia	145 (44.5)	27 (27.8)	118 (51.5)	<0.001
Azotaemia	111 (34.3)	7 (7.3)	104 (45.6)	<0.001
Impaired liver function	220 (67.5)	43 (44.3)	177 (77.3)	<0.001
Acidosis	81 (24.9)	8 (8.3)	73 (32.0)	<0.001
Overall course during hospitalisation				
Shock	156 (48.5)	37 (38.5)	119 (52.7)	0.02
Anaemia	195 (60.0)	37 (38.1)	163 (70.9)	<0.001
Thrombocytopenia	198 (60.9)	48 (49.5)	150 (65.8)	0.001
Azotaemia	129 (39.7)	15 (15.5)	114 (50.0)	<0.001
Hypokalaemia	214 (65.9)	55 (56.7)	159 (69.7)	0.02
Impaired liver function	242 (74.5)	55 (56.7)	187 (82.0)	<0.001
Acidosis	112 (34.5)	13 (13.4)	99 (43.4)	<0.001
Coagulopathy	142 (43.7)	32 (33.0)	110 (48.3)	0.01
GI bleeding	56 (17.2)	5 (5.2)	51 (22.4)	<0.001
Median (IQR) APACHE II score	7 (4-10)	5 (3-8)	8 (5-12)	<0.001§
Median (IQR) SOFA score	5 (2-10)	2 (1-5)	6 (3-11)	<0.001§
Median (IQR) duration of hospitalisation, day	4 (2-6)	3 (2-4)	4 (3-7)	<0.001§
Median (IQR) fever clearance time (FCT), h	33 (16-72)	22 (12-42)	42 (20-92)	<0.001§
No fever or fever cleared before treatment	54 (16.6)	6 (6.2)	48 (21.0)	0.001
Never clear fever before discharge	17 (5.2)	7 (7.2)	10 (4.4)	0.29
Mortality	10 (3.0)	4 (4.1)	6 (2.6)	0.49

‡χ^2 test
§Mann-Whitney U test

4.6 Complications and outcomes of leptospirosis

A total of 10 (3%) patients died from leptospirosis. Median time to death from the onset of illness was 6 days (IQR 3-12 days; range 2-23 days). Eight patients had pulmonary haemorrhage and respiratory failure, 8 patients had renal failure, 8 patients presented to hospital with shock, 8 patients had thrombocytopenia, and 5 patients had jaundice. Clinical details of the 10 fatal cases are shown in **Appendix C**.

Patients with culture-negative leptospirosis were more severely ill than patients with culture-positive infection based on several parameters, including the APACHE II score and SOFA score, both of which reflect disease severity (**Table 4.2**, **4.3** and **4.4**). Neurological and pulmonary complications including aseptic meningitis, convulsion, pulmonary haemorrhage, pulmonary oedema, and respiratory failure were not different between the two groups.

The mortality rate was not different between the two groups. The median (IQR) duration of hospitalisation and fever clearance time were significantly longer in the culture-negative group than the culture-positive group (P=0.0001, both) (**Table 4.4**).

The Kaplan-Meier survival plot between the patients with culture-positive and culture-negative groups is shown in **Figure 4.2**. There was a difference in the survival time between the two groups, in that deaths in the culture-positive group occurred early in the course of the disease (<3 days) whereas the majority of the deaths in the culture-negative group occurred after day 3 of illness.

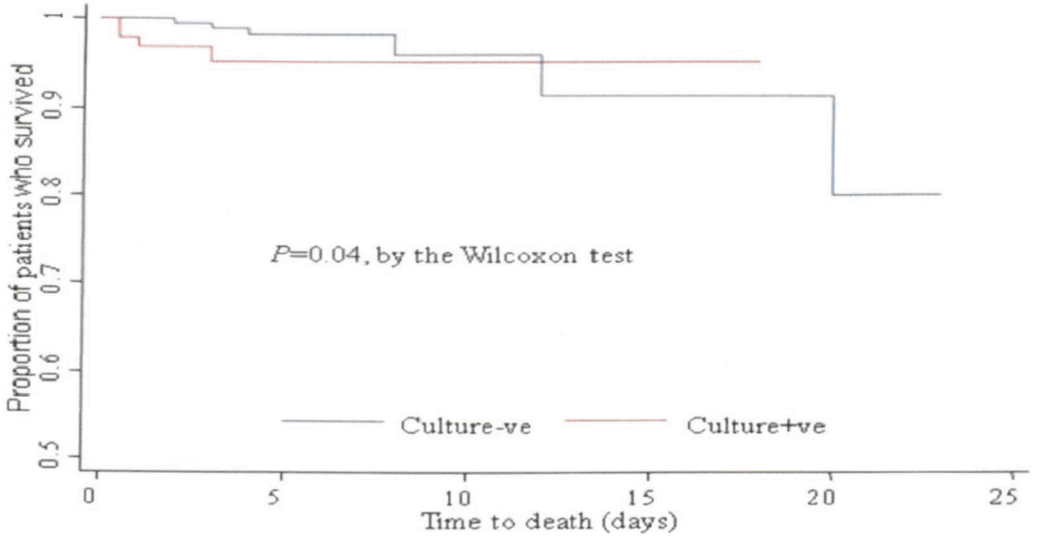

Figure 4.2 Kaplan-Meier survival plot for patients with culture-positive or culture-negative leptospirosis

4.6.1 Predictors for fatal outcome

A comparison of clinical manifestations in survivors and those patients who died from leptospirosis is shown in **Table 4.5**. Bilirubin levels were not significantly different between the patients who died and those who survived. Jaundice occurred in 34.9% of patients who survived and 55.6% of patients who died (P=0.29, Fisher's exact test).

Table 4.5 Comparison of clinical manifestations in survivors and non-survivors of leptospirosis

Parameters	Median (IQR)		*P*-value§
	Survivors (n=321)	Death (n=10)	
Potassium (mmol/L), median (IQR)	3.6 (3.3-3.9)	4.2 (2.9-4.6)	0.31
Bicarbonate (mmol/L), median (IQR)	23 (21-26)	21 (17-23)	0.06
Blood urea nitrogen (mg/dL), median (IQR)	22 (14-45)	81 (45-90)	0.001
Creatinine (mg/dL), median (IQR)	1.5 (1.2-3.6)	5.6 (4.8-6.5)	0.002
Total bilirubin (mg/dL), median (IQR)	1.4 (0.7-4.0)	4.1 (1.4-18.7)	0.07
Direct bilirubin (mg/dL), median (IQR)	0.9 (0.4-2.7)	2.4 (1.2-12.4)	0.05
Aspartate aminotransferase (U/L), median (IQR)	60 (40-107)	129 (119-176)	0.01
Alkaline phosphatase (U/L), median (IQR)	119 (83-172)	185 (159-279)	0.01
Albumin (g/dL), median (IQR)	3.5 (3.0-4.0)	3.1 (2.9-3.3)	0.04
Haemoglobin (g/dL), median (IQR)	12.2 (10.7-13.7)	10.5 (8.4-11.3)	0.003
Platelets ($\times 10^9$/mL), median (IQR)	124 (53-193)	45 (24-73)	0.02
Median (IQR) APACHE II score	7 (4-10)	12 (10-16)	<0.001
Median (IQR) SOFA score	4 (2-6)	14 (12-15)	<0.001
	Number of cases (%)		*P*-value†
On admission			
Shock	116 (37.1)	7 (70.0)	0.05
Haemoptysis	31 (9.7)	5 (50.0)	0.002
Diarrhoea	56 (17.7)	5 (50.0)	0.02
Anaemia	133 (42.1)	10 (100.0)	<0.001
Thrombocytopenia	138 (43.7)	8 (80.0)	0.05
Azotaemia	104 (33.0)	7 (70.0)	0.01
Acidosis	75 (23.8)	6 (60.0)	0.02
Confusion	24 (7.6)	3 (30.0)	0.04
Convulsions	11 (3.5)	2 (20.0)	1.00
Coagulopathy	134 (42.5)	8 (80.0)	0.02
Bleeding diathesis	99 (31.3)	7 (70.0)	0.01
GI bleeding	52 (16.5)	4 (40.0)	0.07
Pulmonary haemorrhage	42 (13.1)	7 (70.0)	<0.001
Respiratory failure	17 (5.3)	8 (80.0)	<0.001
Pulmonary oedema	95 (29.6)	6 (60.0)	0.07

§Mann-Whitney U test
†Fisher's Exact test

In a univariate analysis, the presence of *Leptospira*, hypo- and hyperkalaemia were not associated with a fatal outcome. The presence of shock, haemoptysis, diarrhoea, anaemia, platelet ≤50×109/mL, impaired renal function, especially if creatinine ≥4.0mg/dL, acidosis, ALT ≥300U/L, coagulopathy and abnormal bleeding especially pulmonary haemorrhage but not gastrointestinal bleeding, and respiratory failure were risk factors for death (**Table 4.6**).

Table 4.6 Factors associated with death from leptospirosis

Parameters	OR (95%CI)	*P*-value
Oliguria	9.01 (2.27-35.81)	0.002
Shock	3.96 (1.01-15.62)	0.05
Haemoptysis	9.35 (2.57-34.11)	0.001
Diarrhoea	4.64 (1.30-16.58)	0.02
Anaemia	11.64 (1.46-92.93)	0.02
Thrombocytopenia	5.23 (1.09-25.00)	0.04
Platelet $\leq 50\times10^9$/mL	4.74 (1.30-17.23)	0.02
Azotaemia	7.10 (1.45-34.78)	0.02
Creatinine ≥4.0mg/dL	12.53 (2.54-61.68)	0.002
Acidosis	4.80 (1.32-17.46)	0.02
Hyperkalaemia	5.52 (0.61-50.28)	0.13
Hypoalbuminaemia	7.46 (0.92-60.34)	0.06
Bilirubin ≥5mg/dL	2.80 (0.73-10.71)	0.13
Jaundice	2.33 (0.61-8.85)	0.21
ALT ≥300U/L	19.56 (1.60-238.54)	0.02
AP ≥300U/L	4.45 (0.86-22.91)	0.07
Coagulopathy	5.40 (1.13-25.85)	0.04
Bleeding diathesis	5.11 (1.30-20.19)	0.02
GI bleeding	3.37 (0.92-12.37)	0.07
Pulmonary haemorrhage	15.93 (3.96-64.08)	<0.001
Respiratory failure	71.53 (14.09-363.13)	<0.001

In a multivariable logistic regression model considering age, sex, the presence of oliguria, haemoptysis, diarrhoea, hypotension, hyperkalaemia, hypoalbuminaemia,

platelet $\leq 5\times10^9$/mL, creatinine ≥4mg/dL, anaemia, acidosis, gastrointestinal bleeding, bilirubin ≥5mg/dL, ALT ≥300U/L, AP ≥300U/L, pulmonary haemorrhage, and respiratory failure, the presence of respiratory failure (P=0.01), AP ≥300U/L (P=0.04) and creatinine level ≥4mg/dL (P=0.03) were the only independent factors associated with mortality. (**Table 4.7**) In the final model, the presence of respiratory failure (OR 39.4, 95%CI 7.20-215.05, P<0.001) and a creatininte level ≥4mg/dL (OR 6.2, 95%CI 1.10-34.98, P=0.04) were strongly assocated with a fatal outcome.

Table 4.7 Multivariable logistic regression analysis of independent risk factors for death from leptospirosis

Variable	OR (95%CI)	*P*-value
Oliguria	15.88 (0.44-565.50)	0.13
Shock	1.90 (0.12-30.84)	0.65
Haemoptysis	0.79 (0.03-22.6)	0.89
Diarrhoea	4.91 (0.20-120.08)	0.33
Anaemia	2.03 (0.07-59.96)	0.97
Platelet $\leq 50\times10^9$/mL	0.15 (0.01-3.66)	0.24
Creatinine ≥4.0mg/dL	228.88 (1.97-26,541.54)	0.03
Acidosis	0.11 (0.00-4.56)	0.24
Hyperkalaemia	0.19 (0.00-56.27)	0.57
Hypoalbuminaemia	0.22 (0.01-9.66)	0.44
Bilirubin ≥5mg/dL	0.13 (0.01-1.74)	0.12
ALT ≥300U/L	92.92 (0.00->100,000)	0.44
AP ≥300U/L	41.63 (1.16-1490.66)	0.04
GI bleeding	0.11 (0.01-2.00)	0.14
Pulmonary haemorrhage	0.27 (0.00-15.90)	0.53
Respiratory failure	788.86 (5.77->100,000)	0.01

4.7 Pulmonary manifestations and chest radiographs of patients with leptospirosis

4.7.1 Pulmonary *vs* non-pulmonary leptospirosis

Chest radiographs from 137 patients with leptospirosis and 62 patients with concomitant leptospirosis and scrub typhus infections were available for study. An analysis on pulmonary leptospirosis was only performed on patients with leptospirosis mono-infection, excluding patients with concomitant leptospirosis and scrub typhus infections.

Pulmonary leptospirosis occurred in 92/137 (67.2%) patients. The diagnosis was based on both clinical symptoms and radiographic findings in 57 (62.0%) patients, and on abnormal chest radiographs without respiratory symptoms in 35 (38.0%) patients. The median age, sex, and duration of illness were not significantly different between patients with and without pulmonary involvement.

Respiratory symptoms were commonly present in patients without radiographic changes, and these were similar to patients with CXR changes. Respiratory symptoms represent both upper and lower airway diseases, so a normal CXR with respiratory symptoms in patients with leptospirosis may be due to upper airway irritation or pathology. Moreover, the respiratory symptoms can precede the changes detected on chest radiographs. Haemoptysis occurred in 3 patients without any abnormality on chest radiographs, all of whom subsequently recovered within 3 days and were discharged uneventfully.

There were no significant differences in the bilirubin and all three liver enzyme levels between the pulmonary leptospirosis group and non-pulmonary group. Jaundice was not associated with pulmonary involvement (OR 1.83, 95%CI 0.84-4.01, P=0.13).

Patients with pulmonary leptospirosis had more severe disease than patients with non-pulmonary involvement in terms of significantly higher APACHE II and SOFA scores, longer duration of hospitalisation and longer fever clearance time. Respiratory failure occurred in 17 patients, all of whom were classified as having pulmonary leptospirosis (18.5%). Although the mortality was not significantly different between the two groups, there were no deaths in the non-pulmonary group in this analysis (**Table 4.8**).

Table 4.8 Comparisons of pulmonary and non-pulmonary leptospirosis

Parameters	Median (IQR)			*P*-value§
	All leptospirosis (n=137)	Pulmonary (n=92)	Non-pulmonary (n=45)	
Mean arterial pressure, mmHg	70 (60-83)	70 (60-83)	73 (65-87)	0.05
Respiratory rate, /min	24 (22-26)	24 (22-28)	24 (20-24)	0.02
Haemoglobin, g/dL	12.3 (10.5-13.7)	11.7 (9.9-13.4)	13.0 (11.7-14.2)	0.003
Platelet , $\times 10^9$/mL	117.0 (52.0-185.0)	88.5 (40.5-183.0)	152.0 (91.0-187.0)	0.03
Albumin	3.5 (3.0-3.9)	3.3 (2.9-3.8)	3.8 (3.4-4.2)	<0.001
BUN, mg/dL	23 (14-45)	26 (14-48)	17 (12-33)	0.06
Creatinine, mg/dL	1.6 (1.1-3.8)	1.9 (1.2-4.7)	1.3 (1.0-2.3)	0.02
APACHE II score	8 (4-12)	8 (5-13)	7 (4-10)	0.01
SOFA score	5 (2-10)	6 (3-12)	3 (1-6)	<0.001
Duration of hospitalisation, day	4 (3-6)	5 (3-7)	3 (3-4)	0.002
Fever clearance time, h	32 (14-74)	38 (20-94)	28 (14-52)	0.04
	Number of cases (%)			*P*-value‡
Leptospira isolated	51 (37.2)	29 (31.5)	22 (48.9)	0.05
Respiratory symptoms	80 (58.4)	57 (62.0)	23 (51.1)	0.23
Cough	54 (39.4)	38 (41.3)	16 (35.6)	0.52
Sputum	34 (24.8)	21 (22.8)	13 (28.9)	0.44
Dyspnoea	28 (20.4)	25 (27.2)	3 (6.7)	0.01†
Sore throat	31 (22.6)	20 (21.7)	11 (24.4)	0.72
Haemoptysis	23 (16.8)	20 (21.7)	3 (6.7)	0.03†
Tachypnoea (RR>24/min)	85 (62.0)	61 (66.3)	24 (53.3)	0.14
Anaemia	61 (44.5)	49 (53.3)	12 (26.7)	0.003
Platelet $\leq 50\times 10^9$/mL	34 (24.8)	28 (30.4)	6 (13.3)	0.03
Hypoalbuminaemia	73 (54.1)	58 (64.4)	15 (33.3)	0.001
Severe leptospirosis	96 (70.1)	70 (76.1)	26 (57.8)	0.03
Mortality	7 (5.1)	7 (7.7)	0	0.10†

§Mann-Whitney U test
‡χ^2 test
†Fisher's Exact test

4.7.2 Chest radiographs

Chest radiographs were abnormal in 96 patients. This included 3 patients with isolated cardiomegaly and 1 patient with minimal right pleural effusion without lung parenchymal lesions; these 4 cases were defined as non-pulmonary leptospirosis.

Chest radiograph abnormalities in the remaining 92 patients with pulmonary leptospirosis are summarised in **Table 4.9**. The single most common abnormality in lung parenchyma was diffuse pulmonary infiltrates (86/92 [93.5%]). Cardiomegaly was found in 59/137 (43.1%) patients (including 3 patients in the non-pulmonary group), and 28 (47.5%) of these were associated with increased VPW.

Six patients had a localised lesion without diffuse pulmonary infiltrates, 2 of who also had cardiomegaly. No patients with a localised lesion had a history of haemoptysis or pulmonary haemorrhage as a complication of leptospirosis. Localised lesions were interstitial infiltrate in type for 1 patient; the other 5 patients had nodular or patchy infiltrates, plus confluent consolidation in 3 of these.

Table 4.9 Chest radiograph findings in 92 patients with pulmonary leptospirosis

Findings	Number	%
Diffused pulmonary infiltration	86	62.8
Linear shadow	54	
Nodular or patchy infiltration	35	
Confluent consolidation	12	
Localised lesion	12	8.8
Right upper zone	4	
Right lower zone	6	
Left upper zone	1	
Left lower zone	7	
Minimal pleural effusion	14	15.2
Bilateral	6	
Left side	3	
Right side	5	
Cardiomegaly	55	59.8
Increased vascular pedicle width	33	35.9

4.7.3 Pulmonary haemorrhage in leptospirosis

Pulmonary haemorrhage developed in 31/92 (33.7%) patients with pulmonary leptospirosis, all of whom had diffuse pulmonary infiltrates with 4 patients also having localised confluent consolidation. Nodular or patchy infiltrates were observed in 23 (74.2%) patients, and isolated interstitial infiltrates were present in 6 patients.

Univariate analyses considering the risk factors for having pulmonary haemorrhage are shown in **Table 4.10**. In a multivariable logistic regression model considering age, sex, the presence of oliguria, dyspnoea, tachypnoea, subconjunctival haemorrhage, diarrhoea, gastrointestinal bleeding, hypotension, anaemia, platelet $\leq 100 \times 10^9$/mL, azotaemia, acidosis, hypoalbuminaemia, laboratory coagulopathy, jaundice, and nodular or patchy infiltrations, the presence of nodular or patchy infiltrations (P=0.001), gastrointestinal bleeding (P=0.001), dyspnoea (P=0.01) and hypotension (P=0.04) were associated with pulmonary haemorrhage. In the final model, the presence of dyspnoea (OR 12.08, 95%CI 2.16-67.61, P=0.005), gastrointestinal bleeding (OR 21.84, 95%CI 4.34-109.99, P<0.001), thrombocytopenia (OR 6.96, 95%CI 1.36-35.63, P=0.02), and nodular or patchy infiltrations on chest radiographs (OR 17.29, 95%CI 3.68-81.11, P<0.001) were independent predictors of pulmonary haemorrhage, whereas the presence of shock (P=0.06) was not associated with pulmonary haemorrhage.

Representative examples of chest radiographs of patients with pulmonary haemorrhage are shown in **Appendix D**.

Table 4.10 Factors associated with pulmonary haemorrhage in leptospirosis

Parameters	OR (95%CI)	*P*-value
Oliguria	7.98 (3.23-19.70)	<0.001
Dyspnoea	6.73 (2.81-16.09)	<0.001
Tachypnoea	4.14 (1.48-11.61)	0.01
Subconjunctival haemorrhage	3.14 (1.06-9.29)	0.04
Shock	3.00 (1.30-6.91)	0.01
Diarrhoea	2.69 (1.10-6.57)	0.03
Anaemia	6.39 (2.52-16.24)	<0.001
Thrombocytopenia	17.41 (4.96-61.11)	<0.001
Azotaemia	5.85 (2.44-14.05)	<0.001
Creatinine level ≥4mg/dL	5.98 (2.47-14.50)	<0.001
Acidosis	3.12 (1.36-7.15)	0.01
Hypoalbuminaemia	4.57 (1.73-12.10)	0.002
Jaundice	8.36 (3.32-21.02)	<0.001
Coagulopathy	2.28 (1.01-5.16)	0.05
Gastrointestinal bleeding	15.20 (5.75-40.21)	<0.001
Nodular or patchy infiltrations	17.44 (6.60-46.12)	<0.001

4.7.4 Pulmonary oedema in leptospirosis

Eighty-six patients had diffused pulmonary infiltrates that were compatible with pulmonary oedema. There were attributed to hydrostatic pulmonary oedema (high CT ratio with high VPW) in 25 (29.1%) patients, permeability pulmonary oedema (normal CT ratio with normal VPW) in 26 (30.2%) patients, and undetermined in 35 (40.7%) patients (cardiomegaly without increased VPW [n=28], increased VPW without cardiomegaly [n=7]). Representative examples of chest radiographs of patients with pulmonary oedema are shown in **Appendix E**.

4.7.5 Outcomes and pulmonary involvement in leptospirosis

The presence of pulmonary oedema (OR 3.75, 95%CI 0.44-32.07, *P*=0.23) or cardiomegaly (OR 1.82, 95%CI 0.39-8.45, *P*=0.45) were not associated with mortality. There were 7 deaths in this study, 6 of who had pulmonary haemorrhage. Pulmonary haemorrhage was strongly associated with a fatal outcome (OR 25.20, 95%CI 2.90-218.83, *P*=0.003).

4.8 *Coagulopathies and DIC in leptospirosis*

4.8.1 Patients

Seventy-nine consecutive patients with confirmed leptospirosis were enrolled into the coagulation study. Of these, 48 (61%) were diagnosed by isolation of *Leptospira* from either blood or CSF (45 from blood, 2 from blood and CSF, 1 from CSF only), and 31 (39%) patients were culture-negative and were diagnosed based on the MAT. The serovar (sv.) determinations for cultured *Leptospira* were as follows: *L. interrogans* sv. Autumnalis (32), *L. interrogans* sv. Pyrogenes (3), *L. interrogans* sv. Javanica (1), *L. interrogans* sv. Medanensis (2), and unidentified serovars (10). A summary of baseline clinical features and baseline laboratory results were similar to the whole group of patients with leptospirosis (**Table 4.11** and **4.12**). There were two deaths (2.5%) from pulmonary haemorrhage in the *Leptospira* culture-positive group.

Table 4.11 Baseline information on 79 patients with leptospirosis

	All leptospirosis (n=79) Median (range)
Duration of symptoms (days)	4 (2-8)
Blood urea nitrogen (mmol/L)	7.9 (2.5-36.4)
Serum creatinine (μmol/L)	133 (62-955)
Serum bicarbonate (mmol/L)	23 (11-31)
Total serum bilirubin (μmol/L)	24 (5-397)
Direct serum bilirubin (μmol/L)	14 (3.4-243)
Aspartate aminotransferase (U/L)	61 (13-326)
Albumin (g/L)	36 (20-48)
Globulin (g/L)	30 (19-54)
Haemoglobin (mmol/L)	129 (70-168)

Table 4.12 Severity and outcome of patients with leptospirosis comparing culture-positive and culture-negative groups

	Number of patients (%)			*P*-value
	All leptospirosis (n=79)	Culture+ve (n=48)	Culture-ve (n=31)	
APACHE II score [median (IQR)]	7 (4-10)	5 (3-8)	9 (4-15)	0.001
SOFA score [median (IQR)]	3 (1-11)	2 (1-4)	12 (6-14)	<0.001
Bleeding complication	18 (23)	5 (10)	13 (42)	0.003
Skin/mucosal bleeding	10 (13)	2 (4)	8 (26)	0.01
Gastrointestinal bleeding	7 (9)	1 (2)	6 (19)	0.01
Pulmonary haemorrhage	4 (5)	2 (4)	2 (7)	0.14
Urinary tract bleeding	1 (1.3)	1 (2)	0	1.00
Thrombocytopenia				
Platelet $\leq 100 \times 10^9$/L	30 (38)	10 (21)	20 (65)	<0.001
Platelet $\leq 50 \times 10^9$/L	19 (24)	2 (4)	17 (55)	<0.001
Overt-DIC score [median (IQR)]	4 (3-5)	4 (3-5)	5 (4-6)	0.002
Overt-DIC score ≥ 5	36 (46)	16 (33)	20 (65)	0.01
Died	2 (2.5)	2 (4.2)	0	1.00

4.8.2 Platelets, coagulation tests, DIC score and clinical severity

4.8.2.1 Platelets and coagulation tests

Leptospirosis patients had a significantly lower median (IQR) platelet count than the control group (142.5 (50-211) *vs* 289 (228-328) $\times 10^9$/mL, $P<0.0001$). They also had significantly longer median PTs and APTTs, and higher median levels of D-dimer, F1+2, and TAT ($P<0.0001$) (**Figure 4.3**). These differences were preserved in subgroup analyses, in which culture-negative and culture-positive groups were compared separately with the control group.

4.8.2.2 Coagulation, DIC score and clinical severity

Forty-five (57%) patients presented with severe complications of leptospirosis [oliguria or renal failure (n=24), jaundice (n=26), abnormal bleeding (n=18), hypotension (n=23), respiratory distress or failure (n=6)]. These patients had a significantly lower median (IQR) platelet concentration than the less severe patients

(66.5 (33.5-166) *vs* 192 (144-240) $\times 10^9$/mL, respectively, $P < 0.0001$), but the other coagulation tests and factors were not different between the two groups (data not shown). The platelet count was correlated negatively with the APACHE II and SOFA scores ($|\rho| = 0.53$, $P < 0.001$ for APACHE II score; $|\rho| = 0.75$, $P < 0.001$ for SOFA score), whereas the other coagulation parameters had no significant correlations with APACHE II and SOFA scores. The median (IQR) overt-DIC score in patients with severe leptospirosis was significantly higher than in those patients with less severe disease (6 (5-6) *vs* 4 (3-5), respectively, $P=0.003$). Thirty-six (46%) patients had an overt-DIC score ≥ 5, which is compatible with DIC. The proportion with an overt-DIC score ≥ 5 was higher in patients who had severe disease compared with patients who had less severe disease (27/45 (60%) *vs* 9/34 (26%), respectively, $P=0.003$). In a logistic regression analysis considering platelet count, D-dimer, F1+2, and TAT, platelet count was the only factor independently associated with clinical bleeding ($P=0.003$).

4.8.2.3 Coagulation, DIC score and clinical bleeding

Eighteen patients had clinically significant bleeding. The median APACHE II, SOFA, and overt-DIC scores were higher in patients who bled compared with those who did not (median (IQR); 10 (7-15) *vs* 5 (3-9), respectively, $P=0.0035$ for APACHE II score; 14 (10-16) *vs* 2 (1-6) $P < 0.0001$ for SOFA score; 5 (5-6) *vs* 4 (3-5), respectively, $P=0.0006$ for overt-DIC score). Patients with clinical bleeding had significantly lower platelet counts when compared to the non-bleeding group (median; 43.5 *vs* 162 $\times 10^9$/mL, respectively, $P=0.0002$). The clinical bleeding group also had a higher proportion of patients with thrombocytopenia (14/18 (78%) *vs* 16/61 (27%), $P<0.001$). The results of the other coagulation tests and markers were not different between the patients who had bleeding and those who did not ($P>0.15$). There was no difference in mortality between the two groups (1 (5.6%) in the bleeding group and 1

(1.6%) in the non-bleeding group, P=0.41). The proportion of patients with an overt-DIC score ≥5 was higher for patients who had clinical bleeding than those with no clinical bleeding (14/18 (78%) *vs* 22/61 (36%), respectively, P=0.002).

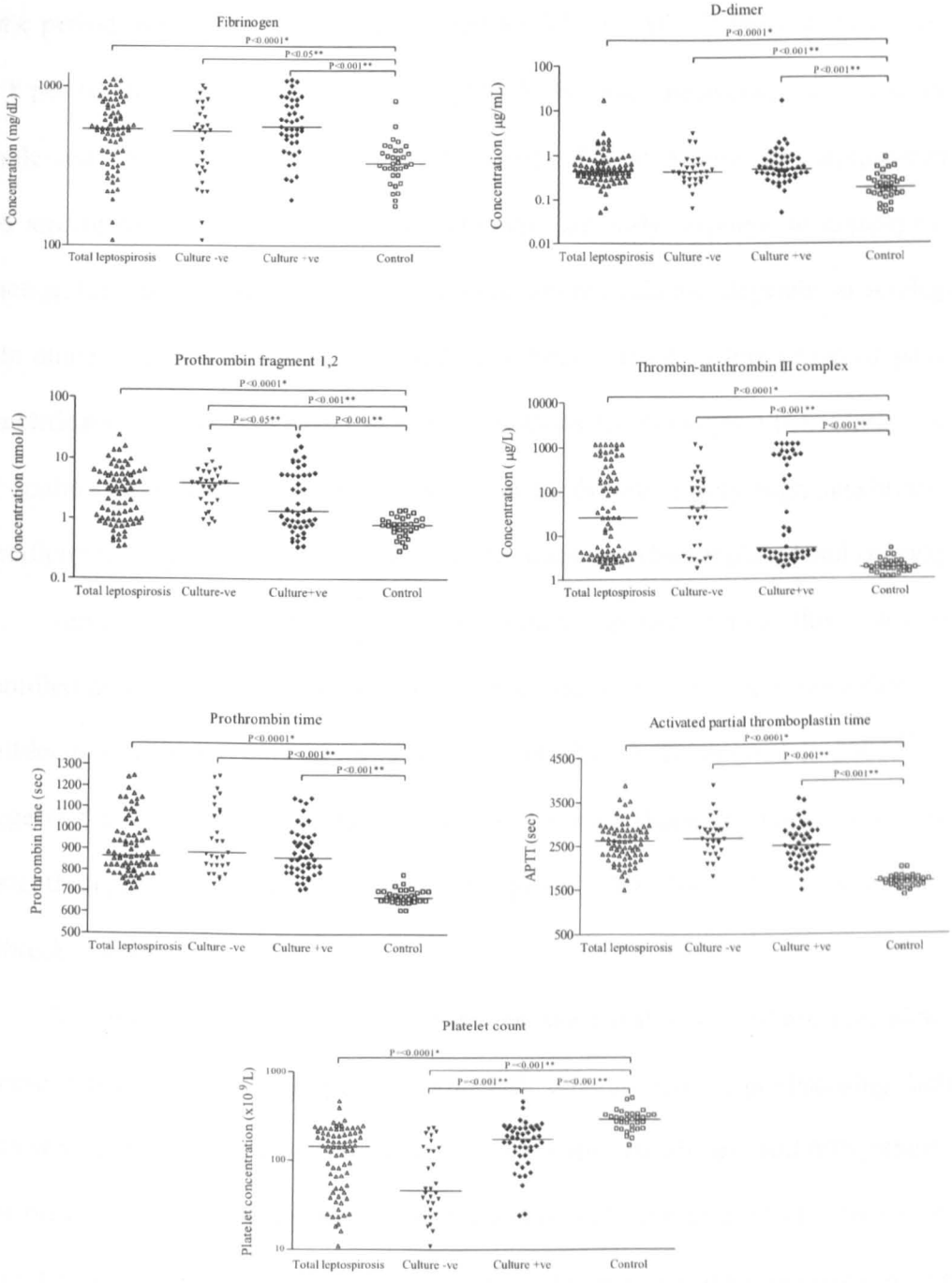

Figure 4.3 Platelet count and coagulation factors in leptospirosis patients and healthy Thai controls

4.9 Discussion

Leptospirosis accounted for 26.5% of AFI in this study conducted in Udon Thani. This is lower than the rates reported for 5 other hospitals in Thailand for the same period, where leptospirosis accounted for 37% of AFI.[261] Disease incidence was 10.8 per 100,000 population, which is slightly higher than the average incidence for the whole country (5-8/100,000 population).[5] Nearly 30% of patients had leptospiraemia, and among these nearly half had no detectable antibody response to *Leptospira*. In practice, laboratory diagnosis of leptospirosis, where available, depends on serological tests alone, and so the diagnosis would have been missed in this group of patients. Simplification and improvement of culture methods for *Leptospira* is urgently needed for routine laboratory diagnosis. Molecular methods with a very high sensitivity and specificity may be a better alternative, if the technique can be simplified and is cheap.

Seventy-six percent of *Leptospira* strains isolated during this study were identified as *L. interrogans* sv. Autumnalis that belonged to a single clone defined by multilocus sequence typing as sequence type 34 (ST34), as reported in 2007.[273] This suggests that there was a sustained outbreak due to a single, biologically successful clone throughout the northeast during the period 1997-2002. The reason for this outbreak is unknown.

The majority of cases with leptospirosis were males of working age, although disease can occur in healthy persons who lack obvious risk of predisposing factors. Almost all patients with leptospirosis had a clear exposure history, and 60% reported a skin break or wound. More than 70% of patients had been in contact with water for more than two hours, when infection can occur in the absence of an obvious skin break. The techniques employed during rice farm in Thailand are based on manual labour and the use of hand held instruments, together with walking barefooted in flooded paddy

fields. Although mechanisation would reduce contact with water, this would require significant financial investment as well as a change in cultural habits and beliefs.

4.9.1 Clinical manifestations, laboratory findings and outcome from leptospirosis

Fever, headache and myalgia were the prominent clinical symptoms associated with leptospirosis, which is consistent with the literature and textbook description. However, the onset and severity of headache were different from that described in the literature since most patients had insidious onset and moderate headache, rather than a sudden severe headache.

The data collected was not designed to describe the pattern of fever for each patient, but a distributional plot of admission body temperature versus duration of symptoms showed a dip in temperature by the time the patient had ill for 7 days. Some patients who presented after more than 7 days of symptoms appeared to have a rising temperature, but there were only 25 (8%) patients in this group (**Figure 4.1**). This may represent the biphasic pattern of fever described for patients with leptospirosis.

Patients with complications such as jaundice, renal failure and thrombocytopenia had a significantly longer duration of symptoms before admission ($P<0.001$), but in reality this represented a median difference of just one day (median [IQR]: 5 [4-6] and 4 [3-5] days for those with and without one or more complications, respectively). There was no difference in the duration of symptoms between patients with or without haemoptysis or pulmonary haemorrhage (data not shown). The majority of patients with complications came to the hospital within the first week in which the immune phase, as described in the literature, should not have been reached.

Icteric leptospirosis occurred in one-third of patients in this study, which is more frequent than previously described.[27,168] Although the number of deaths among patients

with icteric leptospirosis was two times more than those in non-icteric group (4.4% *vs* 1.9%, respectively), this did not reach statistical significance (P=0.29, Fisher's exact), and was not an independent risk factor for fatal outcome. This finding contrasts with the evidence described in the literature and textbooks. The reason why icteric leptospirosis has been strongly associated with death in previous studies may relate to bias in case selection, or perhaps even strain differences and strain-specific manifestations and pathogenicity. The association of male sex to severe disease or death reported from Germany,[124] was not confirmed in this study.

The presence of respiratory failure and acute renal failure (creatinine level ≥4mg/dL) were the only independent prognostic factors for death in this study. This contrasts to previous reports from Brazil in 1999 and Thailand in 2002, in which the only independent risk factor for death was the presence of oliguria in the first report,[69] and oliguria, hyperkalaemia, the presence of pulmonary rales, and shock in the second report.[207] In this study, patients with oliguria were 9 times more likely to die in the univariate analysis, but this factor was not significant in the multivariable analysis. The report from Brazil was performed in a very selective group of patients with acute renal failure, so the renal failure itself was not been evaluated in the study.[69] When this was taken into account, oliguria was usually associated with renal failure, and the presence of renal failure was a stronger association with death rather than oliguria itself.

The presence of hyperkalaemia could not be confirmed as an independent predictor of death in this study, although this was reported from Khon Kaen, northeast Thailand in 2002, and in 2 studies form Turkey published in 2004.[43,89,207] The tests used to confirm the diagnosis of leptospirosis in the study conducted in Khon Kaen and in one of the studies from Turkey were MAT combined with other tests which are not the reference techniques, and leptospirosis could be confirmed by MAT in only 35/121 (28.9%) and 43/72 (59.7%) patients using MAT from Khon Kaen[207] and Turkey,[89]

respectively. The criteria for the diagnosis of leptospirosis were not mentioned in the second report from Turkey.[43] The reported mortality in the Khon Kaen study was very high (14%) when compared with the mortality in other regions of Thailand and neighbourhood countries (<5%).[207] It was difficult, therefore, to compare the result with these studies.

4.9.2 Culture-positive and culture-negative leptospirosis

Patients with blood cultures positive for *Leptospira* presented earlier in the course of their illness than those with negative cultures. This may partly explain why as a group, patients with culture-negative *Leptospira* were more severe in terms of significantly higher APACHE II and SOFA scores, and had significantly more derangement of all laboratory parameters. Although complications occurred more commonly in the culture-negative group, the mortality rate was not difference between the two groups, and the mortality rate was low among the population as a whole. Deaths among those patients with culture-positive infection (4 patients) occurred very early in the course of disease. Three of these patients died within 24 hours of admission or within 72 hours after the onset of illness, and one patient died on day 3 of admission (day 6 of illness). These observations emphasise the fact that severe and fatal leptospirosis can occur in the leptospiraemic phase, when the 'immune phase' has not yet to reach its peak (all 4 deaths had negative MAT). One of the explanations for death in this group of patients may due to a very high bacterial load in those patients. This is supported by the result of the quantitative real time PCR based on 16S rRNA of *Leptospira* performed in one patient in this group who yielded a very high copy number of organisms (nearly 300,000) (personal communication, Dr Janjira Thaipadungpanit). These clinically important differences between culture-positive and culture-negative leptospirosis patients have not been described previously.

One explanation for the finding that nearly half of patients who had culture-positive did not develop an antibody response is that early antibiotic treatment may abrogate the immune response. Data on antimicrobial therapy were not presented in this study since this fell outside the original objectives. However, almost all patients received penicillin G sodium, oral or intravenous doxycycline or cefotaxime therapy during this period.

4.9.3 Pulmonary leptospirosis

Every patient recruited into the study had chest radiograph performed, but only 199/331(60%) patients had a chest radiograph that could be located for review. Pulmonary involvement occurred in almost 70% of patients with leptospirosis in this study, which is similar to the highest rates reported in the literature.[200] Thirty eight percent of pulmonary leptospirosis presented with no respiratory symptoms, and half of non-pulmonary leptospirosis as defined by a normal chest radiograph presented with respiratory symptoms. Thus, chest radiographs are useful in patients with leptospirosis but do not appear to exclude pulmonary involvement.

Patients with pulmonary involvement were more likely to have other complications of leptospirosis including anaemia, thrombocytopenia and the presence of shock and gastrointestinal bleeding, but pulmonary involvement was not associated with jaundice and renal failure. Considered the subset of patients with pulmonary haemorrhage, patients with dyspnoea, gastrointestinal bleeding, thrombocytopenia and nodular or patchy infiltrations on chest radiographs were 12, 22, 7, and 17 times more likely to have pulmonary haemorrhage than patients without these factors. This confirms the findings of previous reports that severe pulmonary involvement in leptospirosis is not associated with the presence of jaundice.[164,212,280] An outbreak of leptospirosis in Korea and Nicaragua was associated with many deaths from pulmonary

haemorrhage in anicteric patients.[212,280] This complication is a major contributor to mortality in patients with anicteric leptospirosis. It has been suggested that there may be an association between specific *Leptospira* serovars and pulmonary haemorrhage, as in the report by Park who made this link with *L. interrogans* sv. Icterohaemorrhagiae.[212] There was no significant association between serovars and pulmonary haemorrhage in this study, but the strain collection was heavily biased towards the 'outbreak' clone (ST34), which made up 76% of strains isolated during this study. [273]

4.9.4 Coagulopathy and DIC in leptospirosis

Abnormal bleeding is not uncommon in leptospirosis, and is often present in fatal cases. The most common site is the lung, other complications such as intracerebral haemorrhage occurring only rarely.[98,275] Immunofluorescence studies performed on infected lung tissues in the experimental leptospirosis guinea pig model have shown the presence of IgM, IgG, IgA and C3 along the alveolar basement membrane, suggesting a possible role for an autoimmune process in fatal pulmonary haemorrhage associated with leptospirosis, but not DIC.[324]

Thrombocytopenia is frequently found in leptospirosis but has not previously been correlated directly with a higher incidence of clinical bleeding.[83,194] In this study patients with clinical bleeding had significantly lower platelet counts. The mechanism of thrombocytopenia in leptospirosis is not well defined. Some studies have suggested defects in production due to a direct toxic effect of the organism on the bone marrow.[193,248] Several studies have shown non-immune platelet destruction as an effect of DIC, immune-mediated causes which respond to treatment with methylprednisolone and hydrocortisone,[71] and increased consumption of platelets secondary to activation of vascular endothelium.[194] Recent studies in a guinea pig model also showed evidence of platelet activation as reflected by increased plasma levels of 11-dehydrogenate

thromboxane B2 (11-DH-TXB2) without significant increases of D-dimer, TAT complexes and fibrinogen degradation products and lack of platelet and fibrin thrombin formation, suggesting that in this animal model the mechanism of bleeding is not DIC.[324]

Other thrombo-haemorrhagic syndromes such as haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) can be the causes of thrombocytopenia and have been rarely reported in leptospirosis.[106,116] Characteristic features of these syndromes, thrombocytopenia and renal impairment, were common in this study (38% and 26%, respectively), but neurological symptoms that occur in TTP were found in less than 10% of cases. Unfortunately, the presence of schistocytes in the peripheral blood smear could not be assessed in this study. Although patients with thrombocytopenia had a lower haemoglobin concentration (P=0.002), and higher bilirubin (P<0.001) and aspartate aminotransferase (P=0.004) level than those who had a normal platelet count (data not shown), the presence of microangiopathic haemolytic anaemia could not be confirmed.

Fibrinogen is an acute-phase reactant and plasma levels may be increased or remain normal during infection, despite ongoing consumption in the process of DIC. Hypofibrinogenaemia may occur infrequently in severe cases of DIC and only 28% of cases had a low fibrinogen level in association with DIC in a previous report.[170,171]

A recent review of coagulation disorders and the pathogenesis of leptospirosis concluded that the bleeding tendency is the result of a dysbalance in the haemostatic equilibrium, but the mechanisms for this are unknown. The haemostatic dysbalance may lead to DIC but this needs further prospective study.[295] Using the diagnostic algorithm introduced by the subcommittee on DIC of the ISTH in 2000,[269] nearly half the leptospirosis patients had a DIC score $\geq$5, indicative of overt DIC. The study was

unable to evaluate this DIC score as a predictor of a fatal outcome because of the very low mortality (2.5%) among our study patients.

4.10 Chapter summary

Nearly one-third of patients with AFI in Udon Thani, northeast Thailand were diagnosed as having leptospirosis. Co-infection with scrub typhus was noted in 30% of patients with leptospirosis. The mortality associated with leptospirosis was 3%, mostly due to pulmonary haemorrhage, which can occur both in patients with culture-positive and culture-negative leptospirosis. Patients with culture-positive leptospirosis presented to hospital earlier than patients with culture-negative infection, and they also had less severe clinical manifestations. Activation of the coagulation cascade was common in our patient population with leptospirosis, and this was often associated with DIC which occurred in nearly half of study patients. However, thrombocytopenia was the only haemostasis-related factor independently associated with clinical bleeding, and should be considered as a predictor of severity in patients with leptospirosis. The presence of dyspnoea, gastrointestinal bleeding, thrombocytopenia and nodular or patchy infiltration on chest radiographs were independent risk factors for pulmonary haemorrhage. The presence of respiratory failure and acute renal failure, creatinine level ≥4mg/dL were independent predictors for a fatal outcome.

Chapter V

Result III: Scrub typhus

5.1 Chapter contents

Scrub typhus was the second most common cause of acute febrile illness in patients presenting to Udon Thani hospital during the study period. The clinical spectrum of the disease is somewhat similar to leptospirosis, as well as other infections endemic in the same geographical areas. The aims of the study were to:

1. Describe in detail the clinical manifestations, laboratory findings, and outcomes of patients with scrub typhus.
2. Determine the incidence of pulmonary involvement, and describe the pulmonary manifestations and chest radiographic findings in patients with pulmonary scrub typhus.

Scrub typhus may involve a range of organs including the liver, kidneys, brain, heart and lungs. These will be described in Aim 1. The literature on pulmonary complications of scrub typhus is limited, and so this was specifically selected for more detailed study in Aim 2.

5.2 Patient and methods

5.2.1 Patients

Three hundred and four patients who were recruited into the acute febrile illness study and who were diagnosed as having scrub typhus were included in this analysis. A proportion of patients had an IgG antibody response in association with an undetectable IgM antibody response. This probably reflects reinfection rather than primary infection. A comparative analysis was performed to compare these two groups. A subset of patients (n=119) for whom chest radiographs were available for review were eligible for a detailed analysis of pulmonary manifestations.

5.2.2 Statistical analysis

Statistical analyses were performed as described in **Chapter II**. Univariate logistic regression analyses were performed using variables that were predicted based on a literature review to affect outcome. These included age, sex, whether the diagnosis was based on an IgG- or IgM antibody response, the presence of oliguria, shock, haemoptysis, diarrhoea, anaemia, abnormal bleeding including gastrointestinal bleeding, pulmonary haemorrhage, thrombocytopenia, jaundice, elevated liver enzymes, acidosis, hypo- and hyperkalaemia, hypoalbuminaemia, and renal failure. The full model included all the variables in the univariate analysis that gave a *P*-value ≤ 0.20. The final model was achieved by stepwise forward selection logistic regression model removal of the variable with the highest *P*-value from the model, until no variable could be removed from the model. These same logistic regression analysis methods were used to define risk factors for severe pulmonary haemorrhage.

5.3 Epidemiology of scrub typhus

5.3.1 Diagnosis

Scrub typhus was diagnosed in 304 (24.3%) patients based on the IFA test. Of these, the diagnosis was based on an IgM antibody response in 227 (74.7%) patients, including a four-fold or greater rise in titre (n=131 [43.1%]), or a single titre of ≥1:400 (n=96 [31.6%]); 1:400 (n=5), 1:800 (n=9), 1:1,600 (n=16), 1:3,200 (n=14), ≥1:6,400 (n=52). The diagnosis in the remaining 77 (25.3%) patients was based on an IgG antibody response, including a four-fold or greater rise in titre (n=42 [13.8%]), or a single titre of ≥1:800 (n=35 [11.5%]); 1:800 (n=14), 1:1,600 (n=9), 1:3,200 (n=7), ≥1:6,400 (n=5).

Of these 304 patients with scrub typhus, 115 (37.8%) had a laboratory confirmed diagnosis of concomitant infection due to other pathogens, including leptospirosis (n=101, 33.2%) and bacterial septicaemia (n=10, 3.3%). Details of concomitant diagnoses are shown in **Table 5.1**.

Table 5.1 Diagnosis for 304 patients with scrub typhus

Diagnosis	Number of cases (%)
Scrub typhus alone	189 (62.2)
Scrub typhus plus one additional concurrent infection	109 (35.9)
Leptospirosis	**95 (31.3)**
Murine typhus	**5 (1.6)**
Bacterial septicaemia	**8 (2.6)**
Escherichia coli	2 (0.7)
Acinetobacter baumannii	1 (0.3)
Acinetobacter lwoffii	1 (0.3)
Citrobacter diversus	1 (0.3)
Citrobacter freundii	1 (0.3)
Burkholderia pseudomallei	2 (0.7)
HIV infection (newly diagnosed)	**1 (0.3)**
Scrub typhus plus two additional concurrent infections	6 (2.0)
Leptospirosis and murine typhus	4 (1.3)
Leptospirosis and *Klebsiella pneumoniae* septicaemia	1 (0.3)
Leptospirosis and *Streptococcus* spp. septicaemia	1 (0.3)

5.3.2 Risk factors and history of exposure

A history of contact with natural water sources (rivers, pond, lakes or paddy fields) or animals (cattle, pigs, dogs) was reported by 266 (89.3%) patients. The two most common modes of contact with water were occupational exposure (either rice and/or non-rice farming) (46.6%) and fishing (38.9%). The proportion of patients who reported contact of more than 8 consecutive hours was highest (66/266 [24.8%]), which is comparable to patients with leptospirosis (**Table 3.3**).

Two hundred and thirty-five (78.9%) patients were rice farmers, and 124 (41.6%) patients had a recent (within 2 weeks) history of a cut, wound or penetrating injury to the foot (n=106), leg (n=36), hand (n=14), or arm (n=5). The presence of an open wound that came into contact with natural water was reported by 166 (50.2%) patients (**Table 3.3**). These findings suggested that a history of exposure to water and animals is not limited to leptospirosis. There was no specific question for the exposure to the scrub vegetation so this could not be evaluated as a risk factor for scrub typhus.

5.4 Clinical manifestations and laboratory findings of scrub typhus

The male:female ratio 1.7:1. The median (IQR) age was 45 (31-56) years, and median (IQR) duration of illness before admission was 5 (4-8) days.

5.4.1 Presenting symptoms and signs

Demographic data and clinical symptoms and signs of all patients with scrub typhus are shown in **Table 5.2**. Also included is an analysis of presenting features in relation to whether the diagnosis of scrub typhus was based on a rise in IgM versus IgG titre.

Table 5.2 Demographic data and clinical manifestations of 304 patients with scrub typhus, and a comparison of clinical features in relation to whether the diagnosis was based on a rise in IgM or IgG titre.

Parameters	Median (IQR)			*P*-value§
	All scrub typhus (n=304)	IgM-based (n=227)	IgG-based (n=77)	
Age, year	45 (31-56)	42 (29-53)	52 (39-59)	<0.001
Duration of symptoms, days	5 (4-8)	6 (4-8)	4 (3-7)	<0.001
Temperature, °C	38.2 (37.2-39.2)	38.2 (37.2-39.2)	38.2 (37.1-39.2)	0.74
Highest temperature during admission, °C	39.1 (38.1-39.8)	39.1 (38.1-39.9)	39.1 (38.2-39.7)	0.96
Mean arterial pressure, mmHg	75 (63-87)	74 (64-87)	77 (61-87)	0.83
Respiratory rate, /min	24 (20-24)	24 (20-24)	24 (20-25)	0.90
Pulse rate, /min	90 (80-100)	90 (82-100)	90 (80-100)	0.22
	Number of cases (%)			*P*-value‡
Sex: male	193 (63.5)	147 (64.8)	46 (59.7)	0.43
Fever	297 (99.7)	222 (100.0)	75 (98.7)	0.09
Chills	240 (80.5)	176 (79.3)	64 (84.2)	0.35
Myalgia	265 (88.9)	199 (89.6)	66 (86.8)	0.50
Muscle tenderness	47 (15.9)	33 (15.0)	14 (18.4)	0.48
Tiredness	246 (82.6)	185 (83.3)	61 (80.3)	0.54
Arthralgia	44 (14.8)	34 (15.3)	10 (13.2)	0.65
Wound	112 (37.8)	84 (38.2)	28 (36.8)	0.71
Skin rash	24 (8.1)	21 (9.5)	3 (4.0)	0.13
Eschar	27 (9.1)	21 (9.5)	6 (7.9)	0.67
Subconjunctival haemorrhage	22 (7.4)	16 (7.3)	6 (7.9)	0.86
Conjunctival suffusion	149 (50.3)	117 (53.2)	32 (42.1)	0.10
Lymphadenopathy	26 (8.8)	21 (9.6)	5 (6.6)	0.43
Palpitations	3 (1.0)	2 (0.9)	1 (1.3)	0.75
Chest pain	21 (7.1)	15 (6.8)	6 (7.9)	0.74
Tinnitus	18 (6.0)	13 (5.9)	5 (6.6)	0.82

§Mann-Whitney U test
‡χ^2 test

Table 5.2 (cont.) Demographic data and clinical manifestations of 304 patients with scrub typhus, and a comparison of clinical features in relation to whether the diagnosis was based on a rise in IgM or IgG titre.

Parameters	Number of cases (%)			*P*-value‡
	All scrub typhus (n=304)	IgM-based (n=227)	IgG-based (n=77)	
Respiratory symptoms	156 (52.4)	119 (53.6)	37 (48.7)	0.46
Cough	119 (39.9)	96 (43.2)	23 (30.3)	0.05
Sputum	56 (18.8)	45 (20.3)	11 (14.5)	0.26
Dyspnoea	41 (13.8)	34 (15.3)	7 (9.2)	0.18
Sore throat	56 (18.8)	41 (18.5)	15 (19.7)	0.81
Tachypnoea (RR ≥24/min)	170 (57.1)	129 (58.1)	41 (54.0)	0.53
Jaundice	78 (26.4)	68 (30.9)	10 (13.2)	0.002
Anorexia	190 (63.8)	147 (66.2)	43 (56.6)	0.13
Nausea	185 (62.1)	145 (65.3)	40 (52.6)	0.05
Vomiting	163 (54.7)	128 (57.7)	35 (46.1)	0.08
Diarrhoea	40 (13.4)	33 (14.9)	7 (9.2)	0.21
Abdominal pain	103 (34.6)	78 (35.1)	25 (32.9)	0.72
Headache	248 (83.2)	187 (84.2)	61 (80.3)	0.42
Confusion	28 (9.4)	16 (7.2)	12 (15.8)	0.03
Deterioration of consciousness	21 (7.1)	13 (5.9)	8 (10.5)	0.17
Convulsions	13 (4.4)	9 (4.1)	4 (5.3)	0.66
Stiff neck	50 (16.9)	37 (16.8)	13 (17.1)	0.90
Oliguria/anuria	52 (17.5)	44 (19.8)	8 (10.5)	0.07
Abnormal bleeding	64 (21.5)	53 (23.9)	11 (14.5)	0.09
Epistaxis	12 (4.1)	10 (4.6)	2 (2.6)	0.47
Haemoptysis	12 (4.0)	11 (4.9)	1 (1.3)	0.17
Petechiael haemorrhage	16 (5.4)	13 (5.9)	3 (4.0)	0.51
Haematemesis	5 (1.7)	5 (2.3)	0	0.19
Melaena	21 (7.1)	20 (9.0)	1 (1.3)	0.02
Haematuria	1 (0.3)	0	1 (1.3)	0.09
Vaginal bleeding	4 (1.3)	4 (1.8)	0	0.24

‡χ^2 test

5.4.1.1 Central nervous system involvement

Headache occurred in 248 (83.2%) patients. Patients reported the severity of headache as mild, moderate and severe in 52 (21.0%), 122 (49.2%), and 74 (29.8%) cases, respectively. The onset of headache was insidious in 174 (70.2%) cases, whereas sudden onset of headache occurred in 74 (29.8%) of patients. The pattern of headache was intermittent pain in 208 (84.2%) patients and persistent pain in 39 (15.8%) patients,

and the character of the headache was dull aching pain and throbbing pain in 193 (78.1%) and 54 (21.9%), respectively.

Thirteen (4.4%) patients had one or more seizures, and 28 (9.4%) patients were confused on admission. The median (range) Glasgow Coma Score (GCS) was 15 (3-15). A stiff neck was found in 52 (17.6%) patients, and lumbar puncture was performed in 41 patients. Cerebrospinal fluid (CSF) profiles of these 41 patients are shown in **Appendix B**. The median (IQR) protein and sugar levels in the CSF were 93 (51-129)g/dL and 57 (49-66)mg/dL, respectively. Leucocytes in the CSF ranged from 0 to 850cells/mL, with a median (IQR) leukocyte count of 17 (2-78) cells/mL. The majority of leucocytes were mononuclear cells (median (IQR) 85% (0-99%)).

5.4.1.2 Gastrointestinal system

Abdominal pain occurred in 103 patients. This was generalised (n=34), epigastric (n-=20), right upper quadrant (n=33), right lower quadrant (n=3), left upper quadrant (n=4), left lower quadrant (n=6), paraumbilical (n=5), or suprapubic (n=1). One patient developed peritonitis 5 days after admission due to perforation of duodenal ulcer in the absence of a previous history of acute or chronic peptic or duodenal ulcer.

5.4.1.3 Hepatobiliary system

Jaundice was clinically visible in 78 (26%) patients, and was graded accordingly to the degree of the icterus of the sclera as judged by a single observer as mild in 32 (11.5%) patients, moderate in 21 (7.1%) patients and severe in 23 (7.8%). Hepatomegaly and splenomegaly were detected in 63 (21.1%) and 16 (5.4%) of patients, respectively.

5.4.1.4 Skin lesions

Minor wounds or abrasions were present in 112 (37.8%) patients. Skin rash was reported by 24 (8.1%) patients, but a maculopapular rash was observed on admission in only 9 patients. An eschar was found in 27 (9.1%) patients. The distribution of eschar is shown in **Table 5.3**.

Table 5.3 Distribution of eschar lesion comparing between male and female

Site	No of cases	
	Male	Female
Neck region	1	-
Axillar area	5	4
Back	2	2
Anterior chest	2	-
Paraumbilical	1	-
Frank region	-	2
Groin area	3	2
Perineum/scrotum	1	-
Thigh/leg	1	1

5.4.1.5 Haematological system and bleeding diatheses

Abnormal bleeding was present in 49 (16.5%) patients, of whom 13 had a platelet count of $\geq 150\times10^9$/mL. The most common site of bleeding was the gastrointestinal tract. Petechial haemorrhage occurred in 16 (6.2%) patients including one patient who had a platelet concentration of $\geq 150\times10^9$/mL.

5.4.2 Laboratory findings

The median (IQR) values of laboratory tests on admission are shown in **Table 5.4**. Thrombocytopenia occurred in 84 (28.2%) patients, including 42 (14.1%) patients with a platelet concentration $\leq 50\times10^9$/mL, and 17 (5.7%) patients with severe thrombocytopenia (platelet concentration $\leq 25\times10^9$/mL) (**Table 5.5**).

The median (IQR) BUN:creatinine ratio was 13.0 (10-17). Eighty-four (28.1%) patients had a creatinine level higher than 1.5mg/dL, 52 (17.4%) of which were ≥4mg/dL.

The bilirubin levels of patients with scrub typhus was within the normal limit, whereas AST, ALT and AP were all higher than the upper limit of the normal range (range 5-40, 5-35, and 39-117U/L, respectively). Twelve patients had an AST level of greater than 300U/L, 8 patients had an ALT of above 300U/L, and 17 patients had AP more than 4 times upper limit of the normal range.

Table 5.4 Laboratory findings for 304 patients with scrub typhus

Parameters	Median (IQR)			*P*-value§
	All scrub typhus (n=304)	IgM-based (n=227)	IgG-based (n=77)	
Sodium, mmol/L	139 (135-141)	138 (134-141)	139 (136-141)	0.12
Potassium, mmol/L	3.6 (3.3-4.1)	3.6 (3.3-4.0)	3.7 (3.4-4.3)	0.17
Bicarbonate, mmol/L	23 (21-26)	23 (21-26)	24 (21-27)	0.06
Blood Urea Nitrogen, mg/dL	18 (12-38)	18 (11-43)	19 (13-34)	0.87
Creatinine, mg/dL	1.3 (1.0-2.5)	1.3 (1.0-3.1)	1.3 (1.0-1.9)	0.33
Total bilirubin, mg/dL	1.0 (0.6-2.9)	1.1 (0.6-3.7)	0.9 (0.6-1.8)	0.06
Direct bilirubin, mg/dL	0.6 (0.3-1.7)	0.6 (0.4-2.3)	0.5 (0.3-1.0)	0.01
AST, U/L	90 (54-148)	91 (56-151)	82 (46-137)	0.15
ALT, U/L	70 (43-114)	76 (43-117)	60 (42-90)	0.05
Alkaline phosphatase, U/L	163 (101-257)	172 (113-270)	123 (95-213)	0.002
Lactate dehydrogenase, U/L	373 (264-502)	384 (277-520)	329 (214-423)	0.02
Albumin, g/dL	3.4 (2.9-3.9)	3.4 (2.9-3.9)	3.5 (3.2-3.9)	0.21
Globulin, g/dL	3.0 (2.6-3.4)	3.0 (2.6-3.5)	3.0 (2.6-3.3)	0.51
Haemoglobin, g/dL	12.2 (10.4-13.7)	12.3 (10.5-13.8)	12.1 (10.0-13.5)	0.24
Haematocrit, %	37 (32-41)	37 (32-42)	36 (31-40	0.21
Leukocyte, $\times 10^9$/L	11.0 (8.1-14.2)	10.7 (8.1-13.7)	11.9 (8.2-15.0)	0.39
% Neutrophil	79 (70-86)	79 (70-86)	82 (72-88)	0.14
Platelets, $\times 10^9$/mL	158 (89-222)	155 (85-211)	167 (104-261)	0.06

§Mann-Whitney U test

5.5 IgM-based vs IgG-based diagnosis of scrub typhus

Patients with scrub typhus who were diagnosed based on the IgG titre were older, and had a significantly shorter duration of symptoms before presenting to hospital

than those who were diagnosed by IgM titre. Patients with an IgM-based diagnosed had a significantly higher conjugated bilirubin (P=0.01), ALT (P=0.05), and alkaline phosphatase (P=0.002) levels, and a higher proportion of patients who presented with jaundice (P=0.002) than in the IgG-based group. Renal function was not different between the two groups. Abnormal bleeding occurred more in the IgM-based group than the IgG-based group (18.9% *vs* 9.2%, respectively, P=0.05), especially bleeding in the gastrointestinal tract (13.1% *vs* 4.0%, respectively, P=0.03).

5.6 Complications and outcomes of scrub typhus

The overall mortality rate for patients with scrub typhus was 3.3% (**Table 5.5**). The median time to death from onset of illness was 10.5 days (IQR 5-15 days; range 4-17 days). Multi-organ involvement was common in the 10 patients who died; 5 patients had pulmonary haemorrhage and respiratory failure, 7 patients had renal failure, 7 patients presented to hospital with shock, 5 patients had thrombocytopenia, and 6 patients had jaundice. Two patients who died had convulsions, deterioration of consciousness, encephalopathy without renal failure, jaundice, and pulmonary haemorrhage. Clinical details of all 10 fatal cases are shown in **Appendix C**.

Table 5.5 Complications and outcome of 304 patients with scrub typhus

Complications	Number of cases (%)			*P*-value
	All scrub typhus (n=304)	IgM-based (n=227)	IgG-based (n=77)	
On admission				
Shock	95 (32.1)	68 (30.9)	27 (35.5)	0.46
Anaemia	132 (44.3)	99 (44.6)	33 (43.4)	0.94
Thrombocytopenia	84 (28.2)	67 (30.2)	17 (22.4)	0.16
Azotaemia	78 (26.2)	63 (28.4)	15 (19.7)	0.14
Hypokalaemia	130 (43.8)	99 (44.8)	31 (40.8)	0.54
Impaired liver function	242 (81.2)	193 (86.9)	49 (64.5)	<0.001
Acidosis	59 (19.9)	45 (20.4)	14 (18.4)	0.71
Overall course during hospitalisation				
Shock	127 (42.9)	88 (38.8)	39 (51.3)	0.09
Anaemia	174 (58.6)	131 (59.3)	43 (56.6)	0.79
Thrombocytopenia	100 (33.7)	78 (35.3)	22 (29.0)	0.41
Azotaemia	85 (28.6)	67 (30.3)	18 (23.7)	0.27
Hypokalaemia	178 (59.9)	138 (62.4)	40 (52.6)	0.13
Impaired liver function	251 (84.5)	197 (89.1)	55 (72.4)	<0.001
Acidosis	87 (29.3)	69 (31.2)	19 (25.0)	0.31
Meningism	52 (17.6)	39 (17.7)	13 (17.1)	0.90
Convulsions	12 (4.0)	8 (3.6)	4 (5.3)	0.53
Coagulopathy	114 (38.4)	87 (39.4)	27 (35.5)	0.55
GI bleeding	32 (10.8)	29 (13.1)	3 (4.0)	0.03
Pulmonary haemorrhage	24 (7.9)	20 (8.8)	4 (5.2)	0.31
Respiratory failure	16 (5.3)	12 (5.3)	4 (5.2)	1.00
Pulmonary oedema	90 (29.6)	71 (31.3)	19 (24.7)	0.27
Median (IQR) APACHE II score	7 (4-10)	7 (4-10)	7 (4-11)	0.35
Median (IQR) SOFA score	3 (1-7)	3 (1-8)	3 (1-6)	0.83
Median (IQR) duration of hospitalisation, day	4 (3-6)	4 (3-7)	4 (2-6)	0.24
Median (IQR) fever clearance time (FCT), h	48 (24-90)	48 (23-94)	48 (26-79)	0.65
No fever or fever cleared before treatment	38 (12.8)	27 (12.2)	11 (14.5)	0.60
Never clear fever before discharge	12 (4.0)	7 (3.2)	5 (6.6)	0.19
Mortality	10 (3.3)	6 (2.6)	4 (5.2)	0.28

5.6.1 Predictors for fatal outcome

A comparison of clinical manifestations of survivors and patients who died from scrub typhus is shown in **Table 5.6**. The median age, sex, the presence of diarrhoea, hypokalaemia, hypoalbuminaemia, aseptic meningitis and abnormal bleeding other than

haemoptysis were not different between patients who died and those who survived (data not shown).

Table 5.6 Comparison of clinical manifestations between survivors and deaths among patients with scrub typhus

Parameters	Number of cases (%)		*P*-value†
	Survivors (n=294)	Death (n=10)	
On admission			
Shock	88 (30.8)	7 (70.0)	0.01
Haemoptysis	8 (2.7)	3 (30.0)	0.004
Jaundice	72 (25.2)	6 (60.0)	0.02
Anaemia	129 (44.9)	8 (80.0)	0.03
Thrombocytopenia	75 (26.0)	6 (60.0)	0.03
Azotaemia	71 (24.7)	7 (70.0)	0.004
Acidosis	53 (18.5)	6 (60.0)	0.01
Confusion	23 (7.8)	5 (50.0)	0.001
Convulsions	9 (3.1)	3 (30.0)	0.01
Deterioration of consciousness	17 (5.9)	4 (40.0)	0.003
Meningitis	50 (17.5)	2 (20.0)	0.69
Coagulopathy	106 (36.9)	8 (80.0)	0.02
Bleeding diathesis	58 (20.1)	6 (60.0)	0.01
Pulmonary haemorrhage	20 (6.8)	4 (40.0)	0.01
Respiratory failure	11 (3.7)	5 (50.0)	<0.001
	Median (IQR)		*P*-value§
Blood urea nitrogen (mg/dL)	17 (12-36)	54 (30-88)	0.003
Creatinine (mg/dL)	1.3 (1.0-2.2)	3.7 (1.7-7.8)	0.003
Total bilirubin (mg/dL)	1.0 (0.6-2.6)	4.2 (0.9-8.7)	0.04
Aspartate aminotransferase (U/L)	89 (54-145)	177 (89-226)	0.03
Creatinine phosphokinase, U/L	135 (66-280)	1665 (151-9010)	0.01
CK-MB mass, µg/mL	19.7 (14.7-29.2)	112.2 (20.8-319.6)	0.01
Albumin (g/dL)	3.4 (3.0-3.9)	2.9 (2.1-3.3)	0.02
Haemoglobin (g/dL)	12.3 (10.5-13.7)	11.2 (9.7-11.9)	0.03
APACHE II score	7 (4-10)	13 (10-21)	<0.001
SOFA score	3 (1-7)	14 (6-18)	<0.001

†Fisher's Exact test
§Mann-Whitney U test

5.6.1.1 Univariate analysis

A univariate analysis was performed to define risk factors for a fatal outcome from scrub typhus (**Table 5.7**). The alkaline phosphatase level, which was generally high in most of the patients with scrub typhus, was not a predictor for death.

Table 5.7 Univariate analysis of factors associated with death from scrub typhus

Parameters	OR (95%CI)	*P*-value
Oliguria	7.89 (2.14-29.07)	0.002
Shock	5.25 (1.33-20.78)	0.02
Convulsions	11.91 (2.68-52.99)	0.001
Haemoptysis	23.83 (5.61-101.31)	<0.001
Anaemia	5.12 (1.07-24.51)	0.04
Thrombocytopenia	4.26 (1.17-15.51)	0.03
Platelet $\leq 25\times10^9$/mL	8.39 (1.96-35.95)	0.004
Azotaemia	7.13 (1.80-28.31)	0.01
Creatinine ≥4.0mg/dL	5.15 (1.43-18.49)	0.01
Acidosis	6.62 (1.81-24.30)	0.004
Jaundice	4.36 (1.20-15.87)	0.03
AST ≥300U/L	6.98 (1.31-37.16)	0.02
ALT ≥300U/L	11.79 (2.05-67.71)	0.01
Coagulopathy	6.83 (1.42-32.76)	0.02
Bleeding diatheses	5.95 (1.63-21.77)	0.01
Pulmonary haemorrhage	9.13 (2.38-35.03)	0.001
Respiratory failure	25.73 (6.48-102.07)	<0.001

5.6.1.2 Multiple variable regression analysis

A multiple variable logistic regression model using age, sex, the presence of oliguria, haemoptysis, jaundice, hypotension, convulsions, thrombocytopenia (platelet $\leq 150\times10^9$/mL, creatinine ≥4mg/dL, AST≥300U/L, ALT≥300U/L, anaemia, and acidosis was performed to adjust for interactions between factors for fatal outcome.(**Table 5.8**) In the final model, the presence of convulsions (OR 18.04, 95%CI 3.23-100.8, *P*=0.001), haemoptysis (OR 19.61, 95%CI 3.62-106.26, *P*=0.001) and ALT ≥300U/L (OR 10.39, 95%CI 1.08-99.53, *P*=0.04) were strongly associated with a fatal outcome.

Table 5.8 Predictors for fatal outcome in patients with scrub typhus

Parameters	OR (95%CI)	*P*-value
Oliguria	1.86 (0.22-15.70)	0.57
Shock	3.54 (0.54-23.31)	0.19
Convulsions	31.21 (3.01-324.02)	0.004
Haemoptysis	10.90 (1.04-113.70)	0.046
Anaemia	5.91 (0.60-57.89)	0.13
Thrombocytopenia	0.67 (0.09-4.99)	0.69
Creatinine ≥4.0mg/dL	0.55 (0.04-6.86)	0.64
Acidosis	3.26 (0.38-27.83)	0.28
Jaundice	0.50 (0.05-5.63)	0.58
AST ≥300U/L	0.26 (0.004-18.51)	0.53
ALT ≥300U/L	103.95 (1.36-7,961.07)	0.04

5.7 Pulmonary scrub typhus

5.7.1 Pulmonary *vs* non-pulmonary scrub typhus

An analysis of pulmonary scrub typhus was performed for 119/203 (59%) patients with scrub typhus mono-infection for whom chest radiographs were available for review. Pulmonary scrub typhus occurred in 70/119 (58.8%) patients. The diagnosis of pulmonary involvement was based on clinical symptoms plus radiographic findings in 39 (55.7%) patients, and on abnormal chest radiographs without respiratory symptoms in 31 (44.3%) patients. The median age, sex, and vital signs on admission were not significantly different between the patients with and without pulmonary involvement. A comparison of the clinical features and laboratory investigations in patients with scrub typhus who did or did not have pulmonary involvement are shown in **Table 5.9**. Unlike pulmonary leptospirosis, thrombocytopenia and the degree of renal impairment were not associated with the presence of pulmonary involvement in scrub typhus, whereas the bilirubin level and all three liver enzymes were significantly higher in patients with pulmonary involvement from scrub typhus than the non-pulmonary group. Patients with pulmonary involvement from scrub typhus had a significantly higher APACHE II score than those without pulmonary involvement, but there were no differences in the SOFA score, fever clearance time, duration of hospitalisation and mortality.

Table 5.9 Comparisons of pulmonary and non-pulmonary scrub typhus

Parameters	Median (IQR)			*P*-value§
	All scrub typhus (n=119)	Pulmonary (n=70)	Non-pulmonary (n=49)	
Duration of illness, days	7 (7-9)	7 (5-10)	6 (3-8)	0.02
Haemoglobin (g/dL)	12.5 (10.6-13.8)	11.8 (10.4-13.5)	12.6 (11.3-14.2)	0.05
Total bilirubin (mg/dL)	0.8 (0.6-1.5)	0.9 (0.6-3.0)	0.7 (0.5-0.9)	0.01
AST (U/L)	111 (73-154)	129 (87-178)	95 (61-124)	0.002
Alkaline phosphatase (U/L)	79 (54-115)	91 (59-125)	70 (47-103)	<0.001
BUN	15 (10-27)	16 (10-31)	13 (9-23)	0.04
Albumin	3.5 (2.9-3.8)	3.3 (2.6-3.7)	3.6 (3.3-4.1)	<0.001
APACHE II	6 (4-10)	7 (5-10)	5 (3-9)	0.03
SOFA score	2 (1-6)	3 (1-7)	2 (0-3)	0.06
Fever clearance time, hour	52 (24-98)	59 (30-112)	46 (18-90)	0.08
	Number of case (%)			*P*-value‡
Respiratory symptoms	65 (54.6)	39 (55.7)	26 (53.1)	0.78
Cough	54 (45.4)	31 (44.3)	23(46.9)	0.78
Sputum	25 (21.0)	14 (20.0)	11 (22.5)	0.75
Dyspnoea	13 (10.9)	12 (17.1)	1 (2.0)	0.01†
Sore throat	20 (16.8)	9 (12.9)	11 (22.5)	0.17
Tachypnoea	66 (55.5)	41 (58.6)	25 (51.0)	0.46†
Haemoptysis	3 (2.5)	3 (4.3)	0	0.27†
Meningitis	27 (22.7)	12 (17.1)	15 (30.6)	0.08
Anaemia	51 (42.9)	37 (52.9)	14 (28.6)	0.01
Hypoalbuminaemia	68 (57.1)	47 (67.1)	21 (42.9)	0.01
Alkaline phosphatase ≥300U/L	177 (114-265)	229 (144-333)	137 (91-195)	0.003
Jaundice	20 (16.8)	18 (25.7)	2 (4.1)	0.002
Mortality	5 (4.2)	3 (4.3)	2 (4.1)	0.96†

§Mann-Whitney U test
‡χ^2 test
†Fisher's Exact test

5.7.2 Chest radiographs

The most common lung parenchymal abnormality on chest radiographs of the 70 patients was diffuse pulmonary infiltrates (64/70 [91.4%]). Six patients had localised parenchymal lesion in the absence of diffuse pulmonary infiltrates, and 10 patients had a

localised lesion in association with diffuse pulmonary infiltrates. Cardiomegaly was presented in 56/119 (47.1%) patients, 28 (50.0%) of which were accompanied with increased VPW.

5.7.3 Pulmonary haemorrhage in scrub typhus

Six (5.0%) of the 70 patients with pulmonary involvement had pulmonary haemorrhage. All six had diffuse pulmonary infiltrates on chest radiographs; 3 patients had a diffuse pattern plus areas of confluent consolidation, and 3 patients had a diffuse pattern plus nodular and patchy infiltrations. All 6 patients had a haemoglobin level <10.5g/dL, albumin level <3.0g/dL, and bicarbonate level ≤20mmol/L. Pulmonary haemorrhage was not associated with bleeding from other sites. One of the six patients died from pulmonary haemorrhage (L41 in **Appendix F**). Examples of chest radiographs from patients with pulmonary haemorrhage from scrub typhus are shown in **Appendix F**.

In a univariate analysis, the presence of oliguria (OR 9.27, 95%CI 1.67-51.63, *P*=0.01), dyspnoea (OR 24.74, 95%CI 2.73-223.9, *P*=0.004), thrombocytopenia (OR 6.47, 95%CI 1.19-35.05, *P*=0.03), and acidosis (OR 32.67, 95%CI 3.57-299.19, *P*=0.002) were associated with pulmonary haemorrhage. In the final model of multivariable logistic regression analysis considering age, sex, the presence of oliguria, dyspnoea, azotaemia, laboratory coagulopathy, and the presence of nodular or patchy infiltrations, the presence of dyspnoea (OR 20.02, 95%CI 2.03-197.26, *P*=0.01) was the only independent factor associated with pulmonary haemorrhage.

5.7.4 Diffuse pulmonary lesions in scrub typhus

The causes of diffuse pulmonary infiltrates in 64 patients were considered. These were attributed to hydrostatic pulmonary oedema (high CT ratio with high VPW)

in 25 (39.1%) patients, permeability pulmonary oedema (normal CT ratio with normal VPW) in 16 (25.0%) patients, or of undetermined cause in 23 (35.9%) patients [cardiomegaly without increased VPW (n=19), increased VPW without cardiomegaly (n=4)]. Examples of representative chest radiographs of pulmonary oedema are shown in **Appendix G**.

Univariate logistic regression analysis was performed to determine risk factors for diffuse pulmonary infiltrates (**Table 5.10**). Age, sex, the presence of renal impairment, and bleeding diathesis were not associated with the occurrence of diffuse pulmonary infiltrates. In the final model including age, sex, the presence of gastrointestinal bleeding, oliguria, dyspnoea, thrombocytopenia, hypoalbuminaemia, AST ≥300U/L, AP ≥300U/L, and anaemia, jaundice (OR 3.59, 95%CI 1.07-12.09, *P*=0.04) and anaemia (OR 2.75, 95%CI 1.15-6.57, *P*=0.02) were the only 2 independent factors for diffuse pulmonary infiltrates.

Table 5.10 Factors associated with diffuse pulmonary infiltrates

Parameters	OR (95%CI)	*P*-value
Oliguria	3.60 (0.95-13.64)	0.6
Dyspnoea	3.20 (1.17-8.75)	0.02
Platelet $\leq 100\times10^9$/mL	2.60 (0.86-7.84)	0.09
Hypoalbuminaemia	2.13 (1.02-4.46)	0.05
Jaundice	4.25 (1.33-13.62)	0.02
AST ≥300U/L	5.59 (0.65-47.92)	0.12
AP ≥300U/L	2.30 (0.91-5.81)	0.08
Anaemia	2.94 (1.37-6.30)	0.01

5.7.5 Outcomes and pulmonary involvement in scrub typhus

The presence of diffuse pulmonary infiltrates (OR 1.30, 95%CI 0.21-8.10, *P*=0.78), pulmonary haemorrhage (OR 5.45, 95%CI 0.51-58.16, *P*=0.16) and cardiomegaly (OR 1.73, 95%CI 0.28-10.73, *P*=0.56) were not associated with death in

patients with scrub typhus. This contrasted with leptospirosis in which pulmonary haemorrhage was strongly associated with mortality.

5.8 Discussion

Scrub typhus was the second most common cause of AFI in Udon Thani. It occurred in one quarter of patients, a fourth of who were diagnosed based on IgG antibody titre. A study from Taiwan has described 2 patterns of antibody response in patients with scrub typhus, which have been termed type 1 responder and type 2 responder.[31] Type 1 responders exhibited a rapid rise in IgM titre, after which the IgG titre slowly increased from the end of the second week of illness. Type 2 responders exhibited an IgG response within the first week of illness, and IgM was not always detectable. It has been suggested that the type I response reflects primary infection, and that the type 2 response represents reinfection.[31] Using this classification, one quarter of patients in this study had putative reinfection. The finding here that patients with putative reinfection were significant older than patients with putative primary infection is consistent with previous reports.[31] Overall clinical presentations were not different between the two groups, including the presentation of eschar, lymphadenopathy and rash, which differs from previous reports.[31,311] Patients with reinfection had a significantly shorter duration of illness before admission, a greater proportion of patients with confusion and shock, but a smaller proportion of patients with other complications including jaundice or liver dysfunction and gastrointestinal bleeding. Overall severity was not different between reinfection and primary infection. This study is the second and largest comparison between reinfection and primary infection in patients with scrub typhus.

Nearly 80% of the study population were rice farmers, who often undertake a range of agricultural activities. Prolonged contact with water during rice farming is likely to be important for the development of leptospirosis, while walking in areas of scrub land during travel to the rice field or during other agricultural activities is likely to

be important for the development of scrub typhus, although the utility of direct questioning in relation to exposure to scrub vegetation has not been addressed. Recent insect bites and exposure to chigger mites was not recognised by the majority of patients. In view of the fact that the bite of chigger mite is usually overlooked and gives no skin reaction at the early phase, this issue was not analysed in the current study. The frequency of variable activities during the working day of a farmer is likely to be associated with risk for dual infections.

Fever and headache were prominent clinical features. The majority of patients reported the insidious onset of a moderately severe, intermittent, dull aching headache, which is similar to that reported by patients with leptospirosis, and contrasts with textbook descriptions. Only 30% of patients had a sudden severe headache, the classical textbook pattern. The presence of an eschar lesion is pathognomonic for scrub typhus, but was observed in fewer than 10% of patients. Thus, although a valuable feature when present, its absence does not rule out the diagnosis of scrub typhus in this setting.

The frequency of jaundice in patients with scrub typhus in Thailand has been reported previously as 35%, based on a small number of selected patients.[16] Jaundice has been reported to occur in 40% of patients with scrub typhus in Taiwan,[115,322] which is higher than the rate found here. Jaundice during scrub typhus appears to be uncommon elsewhere,[217,242] although this may be the result of reporting bias. The incidence of jaundice found in this study was similar to that found in patients with leptospirosis. The high percentage of icteric patients in scrub typhus reported in Taiwan and Thailand may due to the coinfection with leptospirosis, since reports of leptospirosis and scrub typhus coinfections are mainly from these two countries.[162,259,305] This will be discussed in more detail in **Chapter VI**.

Renal impairment was noted in 30% of patients, which contrasts to the previous reports of fewer than 10% in Taiwan.[283] Coinfection with leptospirosis reported in 30%

of patients with scrub typhus in this study may go some way to explain the high proportion of patients with renal impairment. However, jaundice or renal failure caused by scrub typhus mono-infection can occur, and the manifestations of scrub typhus can also mimic the classical 'Weil's disease' of leptospirosis.

Liver impairment was prominent, with raised liver enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase) in more than 80% of patients, and nearly 90% with primary infection. These findings emphasise the usefulness of liver function tests when considering the diagnosis of scrub typhus. Bleeding complications due to DIC have been described as a major cause of pathology and death from scrub typhus;[7,18,262] bleeding occurred in around 20% of patients in this study. The majority of bleeding sites were in the gastrointestinal tract, especially in patients with primary infection. Full coagulation studies could not be performed during the study, although samples are available for future testing.

Scrub typhus led to death in 3.3% of patients in this study. It is possible that these deaths were associated with misdiagnosis and inappropriate antibiotic use. Six of the 10 deaths, including two mixed infections, presented with clinical features consistent with 'Weil's disease', all of which were treated as leptospirosis; only one patient received doxycycline on admission. Two of the 4 remaining patients who died from shock, shock with encephalopathy or renal failure received doxycycline (**Appendix C**). These findings highlight the need for prompt and accurate diagnostic measures and an appropriate antibiotic policy to prevent deaths from this curable disease.

Many factors were associated with death in the univariate analysis, but only three independent predictors were identified in the final model of multivariable logistic regression analysis. An important limitation of this analysis was the small number of deaths, which places restrictions on the robustness of the result. However, the difference

in these three clinical features was very marked in patients who died versus those who survived; patients with convulsions, haemoptysis and respiratory failure were 10, 12, and 9 times more likely to have a fatal outcome than those without these factors. Respiratory failure was an independent predictor for death from scrub typhus and leptospirosis. Unfortunately the coagulation study and DIC markers of these patients could not be included in this study due to a limitation of funding. However, the specimens were collected for future analysis.

Findings from the analysis of pulmonary involvement in patients with scrub typhus were quite similar to those for patients with leptospirosis. Although the analysis was performed in only 59% of patients with scrub typhus, selection bias was unlikely since a chest radiograph was performed in every patient regardless of respiratory symptoms. Chest radiographs were abnormal without respiratory symptoms in over 40% of patients, and over half of patients without pulmonary involvement based on radiological ground had respiratory symptoms. This indicates that chest radiographs can be useful in highlighting pulmonary involvement, but that a normal chest radiographs does not exclude pulmonary involvement.

Adult respiratory distress syndrome (ARDS) is a syndrome that is most accurately defined using invasive measurement of pulmonary arterial wedge pressure. This is not possible in the setting in which this study was undertaken, and the frequency of ARDS in this population remains undefined. Diffuse pulmonary infiltrates reflecting pulmonary oedema occurred more commonly than pulmonary haemorrhage. Although the presence of haemoptysis was one of the independent predictors for death from scrub typhus, the presence of pulmonary haemorrhage was not significantly related with death (P=0.16).

5.9 Chapter summary

Scrub typhus was the second most common cause of AFI in Udon Thani. One third of patients with scrub typhus had a second diagnosis of leptospirosis. A misdiagnosis of leptospirosis in patients with severe scrub typhus or scrub typhus and leptospirosis coinfection runs the risk of inappropriate antimicrobial therapy that may lead to death, especially for those patients present with multiorgan dysfunction involving the liver, kidney, CNS, lung and the circulatory system. Elevation of hepatic enzymes may be useful diagnostic indicators in 80%-90% of patients with scrub typhus. An eschar was found in only 10% of patients, so scrub typhus remains a diagnostic possibility in the absence of this lesion. The presence of convulsions, haemoptysis and respiratory failure were poor prognostic factors for the disease.

Putative re-infection occurs in one-forth of patients. These cases often present at an earlier time-point, are older and more confused on admission. Patients with primary infection had more liver derangement and bleeding complications, especially gastrointestinal bleeding.

Chapter VI

Result IV: Comparison & scoring algorithm

6.1 Chapter contents

The clinical manifestations of patients with leptospirosis and scrub typhus were similar and both diseases were endemic in the northeast Thailand. A detailed description of each disease provides a basis on which to undertake a comparison of the two diseases, and determine whether they can be distinguished apart at the bedside. Specific aims in this chapter were to:

1. Compare the clinical manifestations, laboratory findings, and outcomes between the two diseases.
2. Define the individual risk factors for developing either leptospirosis or scrub typhus.
3. Determine the feasibility of a simple scoring system applied at the bedside to distinguish between leptospirosis and scrub typhus.

6.2 Patients and methods

6.2.1 Patients

Study patients were derived from the same cohort of acute febrile illness described in **Chapter III**. Only patients with confirmed leptospirosis or scrub typhus were used in the analyses. Patients with concomitant leptospirosis and scrub typhus infections were treated as a third group.

6.2.2 Data analysis

6.2.2.1 Multivariable logistic regression analysis

All analyses were performed using the STATA/SE statistical software, version 9.0 (StataCorp, Texas, USA). The univariate analysis to determine the potential predictors of leptospirosis or scrub typhus as the cause of acute febrile illness in the cohort were performed using logistic regression modelling. The 45 variables, including demographic data, history of exposure to water, the presence of a wound, clinical symptoms and signs and laboratory parameters on admission were initially selected based on clinical judgement, together with data arising from the in-depth study of each disease. Jaundice was found in this study to be an important clinical feature of leptospirosis, whereas liver enzyme derangement was also important in scrub typhus, so both bilirubin level and enzyme level could not be omitted. ALT is found primarily in the liver, but AST can be found in several tissues, including liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas and lung. ALT elevation indicates hepatocellular damage, while AP elevation suggests cholestasis, which may reflect a different mechanism of pathogenesis. In view of this, both the ALT:bilirubin ratio and AP:bilirubin ratio were calculated and selected for the model. A multivariable logistic regression model using a purposeful selection of covariates method was developed

using all variables which were significant at $P \leq 0.20$. The final model was achieved by removing one variable which had the highest *P*-value (least significant) and *P*-value >0.05 at a time from the model, until no variable could be removed from the model. Variables were successfully removed from the model if the *P*-value as determined by the likelihood ratio test was >0.05. The variables retained in the final model were considered as independent predictors and were further used to develop the scoring algorithm.

6.2.2.2 Scoring algorithm

Variables from the final model of logistic regression analysis were used to construct a scoring algorithm. A numerical value was assigned to each variable based on the digit nearest to the coefficient derived for that factor in the model. The total score for each patient was calculated by the summation of the value for each of these.

6.2.2.3 Bootstrap resampling

Bootstrap resampling analyses were used to assess the internal validity of the model and to adjust for over-fitting or over-optimism. One thousand random bootstrap samples were drawn with replacement from the original data set. The bootstrap sample set were used as a training dataset and the original sample set were test data for the logistic regression model and scoring system generated by the bootstrap sample set. The mean difference between the AUC derived from the bootstrapping and the AUC of the original data set was used to adjust the AUC derived from the final logistic regression model.

6.2.2.4 Score evaluation using second independent database

The LEST score was further evaluated using a second independent database of patients with confirmed leptospirosis and/or scrub typhus admitted to Udon Thani

Regional Hospital during July 2003 and December 2006. These were patients who were recruited during a different study but using the same criteria as those used for the first patient group described in this thesis. The data, specimen collection and laboratory diagnosis for leptospirosis and scrub typhus were exactly the same as the method described in **Chapter II**.

6.3 Comparing leptospirosis and scrub typhus

Comparisons of epidemiological data, clinical and laboratory manifestations were performed between 230 patients with leptospirosis and 203 patients with scrub typhus. Mixed infection was treated as a third group (n=101).

The proportion of patients with a history of penetrating injury (147 (62.1%) *vs* 73(36.3%), $P<0.001$) and history of overall exposure to either water or animals (217 (94.8%) *vs* 175 (87.1%), $P=0.005$) was significantly higher in the leptospirosis group than in the scrub typhus group (**Table 3.3**). The other risk factors and history of individual exposure to water and animals were similar between the two diseases. This emphasised the fact that the two diseases cannot be discriminated using the history of exposure in the area where both diseases are endemic.

Comparisons of demographic data and clinical presentations of patients with leptospirosis and scrub typhus are shown in **Table 6.1**. Although the clinical spectra of the two diseases were similar, numerous parameters were significantly different between the two groups. However, the presence of fever and other constitutional symptoms: headache, alteration of consciousness, rigors, skin rash, arthralgia, palpitations, respiratory symptoms, and gastrointestinal symptoms including anorexia, nausea, vomiting, and abdominal pain were not significantly different between the two groups. The presence of eschar was the only manifestation that gave absolute

discrimination between the two diseases, since it was only found in patients with scrub typhus.

Table 6.1 Clinical manifestations comparing patients with leptospirosis or scrub typhus

Parameters	Median (IQR)			*P*-value§
	Mixed infection (n=101)	Leptospirosis (n=230)	Scrub typhus (n=203)	
Age, y	43 (31-54)	36 (25-46)	45 (31-56)	<0.001
Duration of symptoms, d	5 (4-6)	4 (3-5)	6 (4-8)	<0.001
Temperature, °C	37.5 (36.7-38.7)	38.0 (37.2-39.1)	38.5 (37.6-39.4)	0.03
Highest temperature, °C	38.3 (37.8-39.2)	38.9 (38.0-39.7)	39.3 (38.4-40.0)	0.002
Mean arterial pressure, mmHg	70 (60-80)	73 (62-85)	80 (70-90)	0.01
Respiratory rate, /min	24 (20-26)	24 (20-26)	24 (20-24)	0.03
Pulse rate, /min	94 (84-104)	94 (84-108)	90 (80-100)	0.002
	Number of cases (%)			*P*-value‡
Sex: male	253 (76.4)	175 (76.1)	115 (56.7)	<0.001
Wound	51 (52.6)	141 (62.1)	73 (36.3)	<0.001
Eschar	4 (4.2)	0	23 (11.4)	<0.001
Subconjunctival haemorrhage	14 (14.7)	22 (9.7)	8 (4.0)	0.02
Conjunctival suffusion	66 (69.5)	142 (62.6)	83 (41.3)	<0.001
Myalgia	86 (88.7)	220 (96.1)	179 (89.1)	0.005
Muscle tenderness	22 (23.2)	55 (24.2)	25 (12.4)	0.002
Tiredness	80 (82.5)	209 (91.3)	166 (82.6)	0.01
Chest pain	10 (10.3)	30 (13.1)	11 (5.5)	0.01
Tinnitus	7 (7.2)	5 (2.2)	11 (5.5)	0.07
Respiratory symptoms	57 (58.8)	113 (49.3)	99 (49.3)	0.99
Cough	38 (39.2)	81 (35.4)	81 (40.3)	0.29
Sputum	18 (18.6)	44 (19.2)	38 (18.9)	0.94
Dyspnoea	25 (25.8)	33 (14.4)	16 (8.0)	0.04
Sore throat	20 (20.6)	44 (19.2)	36 (17.9)	0.73
Tachypnoea (RR>24/min)	66 (68.0)	135 (59.0)	104 (51.7)	0.13
Jaundice	48 (49.0)	67 (29.5)	32 (15.9)	0.001
Diarrhoea	20 (20.6)	41 (17.9)	20 (10.0)	0.02
Convulsions	6 (6.2)	2 (0.9)	7 (3.5)	0.06
Meningism	10 (10.5)	21 (9.3)	42 (20.9)	0.001
Oliguria/anuria	30 (30.9)	42 (18.3)	22 (11.0)	0.03
Abnormal bleeding	36 (37.1)	70 (30.6)	28 (13.9)	<0.001
Epistaxis	6 (6.3)	23 (10.1)	6 (3.0)	0.003
Haemoptysis	7 (6.9)	29 (12.6)	5 (2.5)	<0.001
Petechiael haemorrhage	12 (12.6)	8 (3.5)	4 (2.0)	0.33
Haematemesis	3 (3.1)	9 (3.9)	2 (1.0)	0.05
Melaena	14 (14.4)	28 (12.2)	7 (3.5)	0.001
Haematuria	0	5 (2.2)	1 (0.5)	0.14
Vaginal bleeding	1 (1.0)	2 (0.9)	3 (1.5)	0.55

§Mann-Whitney U test; excluding mixed infection group

‡χ^2 test, comparing two groups, excluding mixed infection group

6.3.1 Laboratory findings

Comparisons of laboratory parameters for patients with leptospirosis and scrub typhus are shown in **Table 6.2**.

The median haemoglobin level and white cell count were not significantly different between the leptospirosis and scrub typhus groups. The percentage neutrophil count was higher in the leptospirosis group, while there was a higher proportion of patients with atypical lymphocytes in the scrub typhus group.

The bicarbonate level was not significantly different between the two groups, despite the significant differences in other renal function tests (BUN and creatinine).

Table 6.2 Laboratory findings comparing patients with leptospirosis or scrub typhus

Parameters	Median (IQR)			*P*-value§
	Mixed infection (n=101)	Leptospirosis (n=230)	Scrub typhus (n=203)	
Sodium, mmol/L	139 (135-142)	139 (137-142)	138 (135-141)	0.001
Potassium, mmol/L	3.6 (3.3-4.0)	3.6 (3.2-3.9)	3.7 (3.3-4.1)	0.04
Bicarbonate, mmol/L	22 (19-25)	24 (21-26)	24 (21-26)	0.79
Blood urea nitrogen, mg/dL	35 (18-77)	20 (13-38)	15 (10-24)	<0.001
Creatinine, mg/dL	2.5 (1.3-5.6)	1.4 (1.1-2.6)	1.1 (0.9-1.5)	<0.001
Creatinine phosphokinase, U/L	193 (79-375)	197 (91-528)	126 (64-251)	0.003
CK-MB mass, µg/mL	22.4 (15.7-35.7)	20.1 (15.2-31.0)	19.5 (14.2-27.4)	0.26
Lactate dehydrogenase, U/L	309 (219-461)	247 (197-335)	387 (298-504)	<0.001
Total bilirubin, mg/dL	2.3 (0.9-9.6)	1.4 (0.7-3.1)	0.8 (0.6-1.6)	<0.001
Direct bilirubin, mg/dL	1.5 (0.5-6.6)	0.8 (0.4-2.0)	0.5 (0.3-0.9)	<0.001
AST, U/L	63 (41-134)	59 (40-108)	101 (65-149)	<0.001
ALT, U/L	52 (30-76)	49 (31-74)	81 (51-124)	<0.001
Alkaline phosphatase, U/L	129 (94-184)	114 (82-168)	188 (111-271)	<0.001
ALT:bilirubin ratio	26 (9-93)	62 (20-129)	170 (73-317)	<0.001
AP:bilirubin ratio	88 (26-216)	158 (66-317)	367 (162-627)	<0.001
Albumin, g/dL	3.3 (2.8-3.7)	3.6 (3.1-4.1)	3.5 (3.0-3.9)	0.04
Globulin, g/dL	2.8 (2.4-3.3)	2.8 (2.4-3.1)	3.1 (2.7-3.5)	<0.001
Platelets, $\times 10^9$/mL	91 (42-189)	134 (63-195)	182 (127-251)	<0.001
% Neutrophil	83 (77-88)	85 (79-89)	77 (68-86)	<0.001
Presence of atypical lymphocytes, No (%)	13 (13.4)	19 (8.3)	42 (20.9)	<0.001‡

§Mann-Whitney U test; excluding mixed infection group
‡χ^2 test, comparing two groups, excluding mixed infection group

6.3.2 Complications and outcomes

Patients with leptospirosis had more severe disease than patients with scrub typhus in terms of the proportion with shock, thrombocytopenia, and/or renal impairment on admission. A higher proportion of patients presented with jaundice in the leptospirosis group. There was no significant difference in the proportion of patients with overall liver impairment on admission (defined as hyperbilirubinaemia or elevated liver enzymes), but during admission the liver function in the scrub typhus group became significantly more deranged.

Table 6.3 Complications and outcomes of 534 patients with leptospirosis or scrub typhus

Complications	Number of cases (%)			*P*-value*
	Mixed infection (n=101)	Leptospirosis (n=230)	Scrub typhus (n=203)	
On admission				
Shock	46 (48.4)	77 (33.8)	49 (24.4)	0.03
Thrombocytopenia	65 (66.3)	125 (54.8)	75 (37.5)	<0.001
Azotaemia	48 (49.5)	63 (27.8)	30 (14.9)	0.001
Impaired liver function	64 (65.3)	107 (47.1)	112 (55.7)	0.08
Overall course during hospitalisation				
Shock	55 (57.9)	101 (44.5)	72 (35.8)	0.07
Thrombocytopenia	68 (70.8)	130 (56.8)	81 (40.3)	0.001
Azotaemia	51 (53.1)	78 (34.1)	34 (16.9)	<0.001
Acidosis	46 (47.9)	66 (28.8)	42 (20.9)	0.06
Impaired liver function	79 (82.3)	163 (71.2)	173 (86.1)	<0.001
Coagulopathy	48 (50.0)	94 (41.1)	66 (32.8)	0.08
GI bleeding	22 (22.9)	34 (14.9)	10 (5.0)	0.001
Pulmonary haemorrhage	14 (13.9)	35 (15.2)	10 (4.9)	<0.001
Meningitis	10 (10.5)	21 (9.3)	42 (20.1)	0.001
Median (IQR) APACHE II score	9 (6-11)	6 (3-10)	6 (3-9)	0.61
Median (IQR) SOFA score	7 (3-12)	4 (1-8)	2 (0-5)	<0.001
Median (IQR) duration of hospitalisation, day	4 (3-7)	3 (2-6)	4 (2-6)	0.02
Median (IQR) fever clearance time (FCT), h	38 (10-80)	28 (9-67)	48 (19-96)	<0.001
No fever or fever cleared before treatment	19 (19.6)	35 (15.3)	19 (9.5)	0.07
Never clear fever before discharge	2 (2.1)	15 (6.6)	10 (5.0)	0.49
Mortality	2 (2.0)	8 (3.5)	8 (3.9)	0.80

* Two groups comparison; leptospirosis and scrub typhus, excluding mixed infection group

Bleeding complications were significantly more common in leptospirosis patients, despite the fact that laboratory coagulopathy and anaemia were comparable between the two groups both on admission and during hospital stay. Features of central nervous system involvement were more common in patients with scrub typhus. The APACHE II score suggested that disease severity was comparable for the two diseases, but the SOFA score was significantly higher in patients with leptospirosis. The median duration of hospitalisation and fever clearance time were significantly longer in the scrub typhus group than leptospirosis group, but overall mortality was comparable between the two groups.

Multiple complications could occur in a single patient, as shown in **Figures 6.1 - 6.3** for patients with leptospirosis, scrub typhus and mixed infections, respectively. Jaundice and renal failure occurred in 29.7% and 14.8% of patients with mixed infections and leptospirosis, respectively. These rates were much higher than those for patients with scrub typhus alone (5.5%).

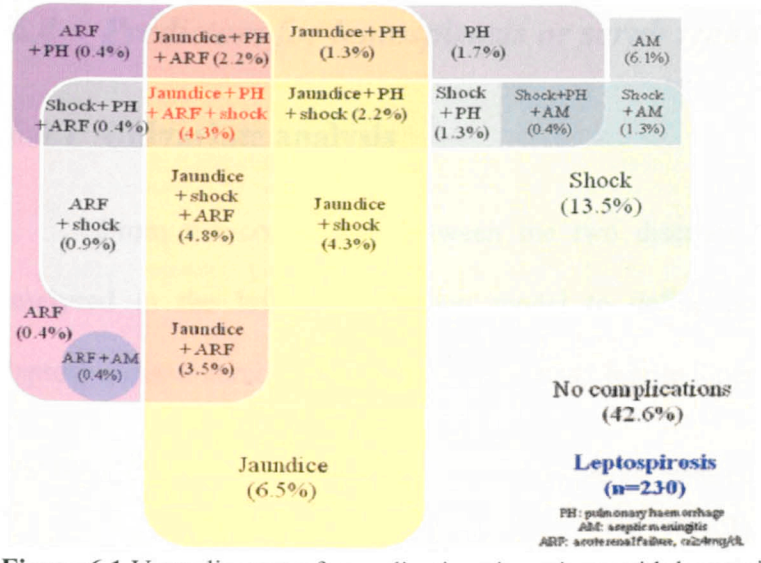

Figure 6.1 Venn diagram of complications in patients with leptospirosis

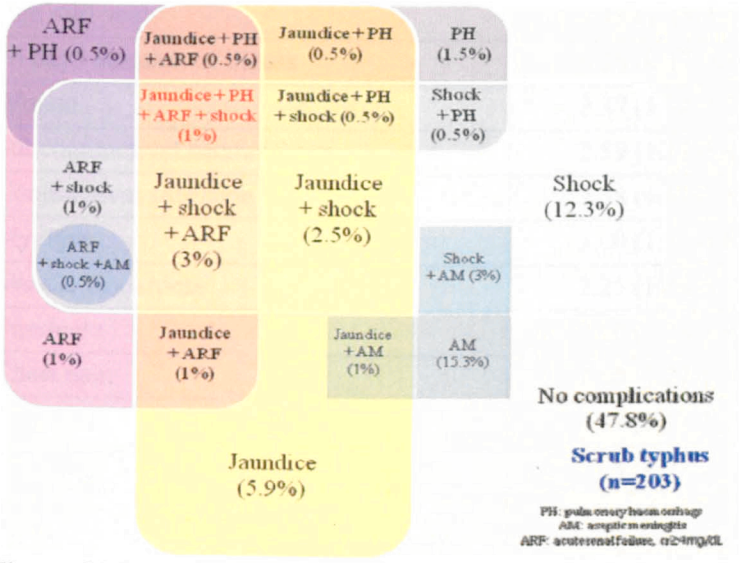

Figure 6.2 Venn diagram of complications in patients with scrub typhus

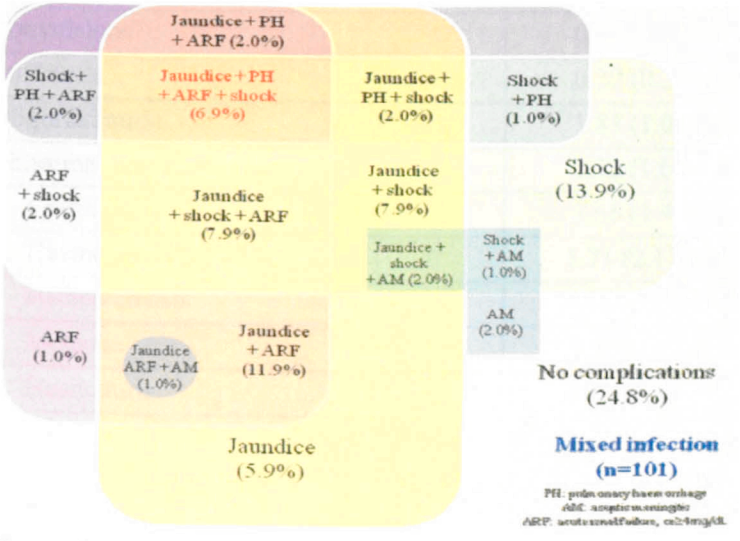

Figure 6.3 Venn diagram of complications in patients with mixed infections

6.4 Predictors for leptospirosis or scrub typhus

6.4.1 Univariate analysis

From the comparison between the two diseases, 45 variables were further analysed in the logistic regression model to define predictors for either having leptospirosis or scrub typhus as a cause of acute febrile illness (**Table 6.4**).

Table 6.4 Univariate analysis of 45 parameters to predict leptospirosis rather than scrub typhus

Parameters	OR (95%CI)	*P*-value[a]
Sex: male	2.43 (1.61-3.67)	<0.001
Age <40 year	2.13 (1.45-3.14)	<0.001
≥5 days duration of illness	0.26 (0.17-0.38)	<0.001
Wound	2.87 (1.94-4.26)	<0.001
Subconjunctival haemorrhage	2.59 (1.13-5.95)	0.03
Conjunctival suffusion	2.38 (1.61-3.50)	<0.001
Myalgia	3.00 (1.35-6.69)	0.01
Muscle tenderness	2.25 (1.34-3.78)	0.002
Tiredness	2.20 (1.23-3.96)	0.01
Chest pain	2.60 (1.27-5.34)	0.01
Tinnitus	0.39 (0.13-1.13)	0.08
Dyspnoea	1.68 (1.02-2.79)	0.04
Tachypnoea	1.34 (0.91-1.96)	0.13
Jaundice	2.23 (1.39-3.57)	0.001
Diarrhoea	1.97 (1.11-3.50)	0.02
Convulsions	0.02 (0.05-1.19)	0.08
Stiff neck	0.39 (0.22-0.68)	0.001
Oliguria/anuria	1.83 (1.05-3.18)	0.03
Abnormal bleeding	2.72 (1.67-4.43)	<0.001
Epistaxis	3.66 (1.46-9.19)	0.01
Haemoptysis	5.71 (2.17-15.06)	<0.001
Haematemesis	4.07 (0.87-19.06)	0.08
Melaena	3.86 (1.65-9.05)	0.002
Haematuria	4.46 (0.52-38.53)	0.17

[a]*P*-values are shown if ≤0.20; ns: not significant

Table 6.4 (cont.) Univariate analysis of 45 parameters to predict leptospirosis rather than scrub typhus

Parameters	OR (95%CI)	*P*-value[a]
Shock	1.58 (1.04-2.41)	0.03
Thrombocytopenia	3.98 (2.49-6.36)	<0.001
Platelet $\leq 50\times10^9$/mL	6.40 (2.95-13.90)	<0.001
Platelet $\leq 25\times10^9$/mL	4.97 (1.68-14.74)	0.004
Acidosis	1.67 (1.02-2.75)	0.04
Anaemia	1.02 (0.70-1.50)	0.91
Azotaemia	2.19 (1.35-3.56)	0.002
Creatinine ≥4mg/dL	2.39 (1.31-4.35)	0.01
Hypoalbuminaemia	0.73 (0.50-1.07)	0.10
Alkaline phosphatase ≥300U/mL	0.21 (0.11-0.40)	<0.001
AST ≥150U/mL	0.34 (0.20-0.58)	<0.001
ALT ≥150U/mL	0.29 (0.14-0.60)	0.001
Total bilirubin ≥5mg/dL	2.06 (1.15-3.69)	0.02
Total bilirubin ≥10mg/dL	3.00 (1.32-6.81)	0.01
ALT:bilirubin ratio ≥60	0.30 (0.20-0.46)	<0.001
AP:bilirubin ratio ≥60	0.23 (0.12-0.45)	<0.001
Impaired liver function	0.71 (0.48-1.04)	0.08
Coagulopathy	1.42 (0.96-2.11)	0.08
Bleeding diathesis	2.72 (1.67-4.43)	<0.001
GI bleeding	3.33 (1.60-6.93)	0.001
Pulmonary haemorrhage	3.35 (1.61-6.97)	0.001
Respiratory failure	1.83 (0.80-4.17)	0.15

[a]*P*-values are shown if ≤0.20; ns: not significant and *P*-value >0.2

6.4.2 Multivariable analysis

A full model of multivariable logistic regression analysis was developed including all of the variables above that showed a *P*-value of ≤0.20 from the univariate

logistic regression model. A purposeful selection multivariable logistic regression analysis was then performed.

Eight parameters were retained in the final model. There were 4 significant independent predictors favouring leptospirosis as a cause of acute febrile illness and 4 predictors in favouring of scrub typhus (**Table 6.5**). The AUC for this model was 0.83, and the Hosmer-Lemeshow goodness-of-fit test was not significant for the lack of fit (Hosmer-Lemeshow statistics =4.42, df=10, P=0.82).

Table 6.5 Multivariable predictors of leptospirosis as a cause of acute febrile illness

Predictors	Coefficient (95%CI)	OR (95%CI)	*P*-value
Age <40 years	0.8 (0.4-1.3)	2.29 (1.42-3.70)	0.001
Duration of symptoms ≥5days	-1.6 (-2.1--1.1)	0.21 (0.13-0.33)	<0.001
The presence or history of penetrating wound	1.2 (0.7-1.7)	3.29 (2.05-5.30)	<0.001
Conjunctival suffusion	0.6 (0.2-1.1)	1.90 (1.19-3.04)	0.01
Tinnitus	-1.3 (-2.6--0.03)	0.27 (0.08-0.97)	0.045
Platelet $\leq 50\times10^9$/mL	1.4 (0.5-2.3)	4.17 (1.72-10.10)	0.002
ALT: direct bilirubin ratio ≥60	-1.0 (-1.5--0.5)	0.37 (0.22-0.62)	<0.001
Meningitis	-0.9 (-1.6--0.2)	0.39 (0.19-0.79)	0.01

Another multivariable logistic regression model using the same variables were also tested to check the robustness of the methodology selected in this study. Both forward and backward stepwise selection multivariable logistic regression models yielded the same 8 variables with very similar coefficients as the independent predictors for diagnosing leptospirosis. Thus, the points for each variable use in the score were the same, regardless of the analytical model used.

6.5 Scoring algorithm for predicting leptospirosis or scrub typhus

6.5.1 LEptospirosis-Scrub Typhus (LEST) score

A scoring algorithm was constructed based on the combination of 8 predictors of leptospirosis and scrub typhus in the final multivariable logistic regression model. The coefficient (95% CI) for each variable in the final model was plotted, as shown in **Figure 6.4**. Predictors associated with leptospirosis were assigned a positive numerical value, and predictors associated with scrub typhus were assigned a negative numerical. The final score for each patient was reached by adding together all of the points.

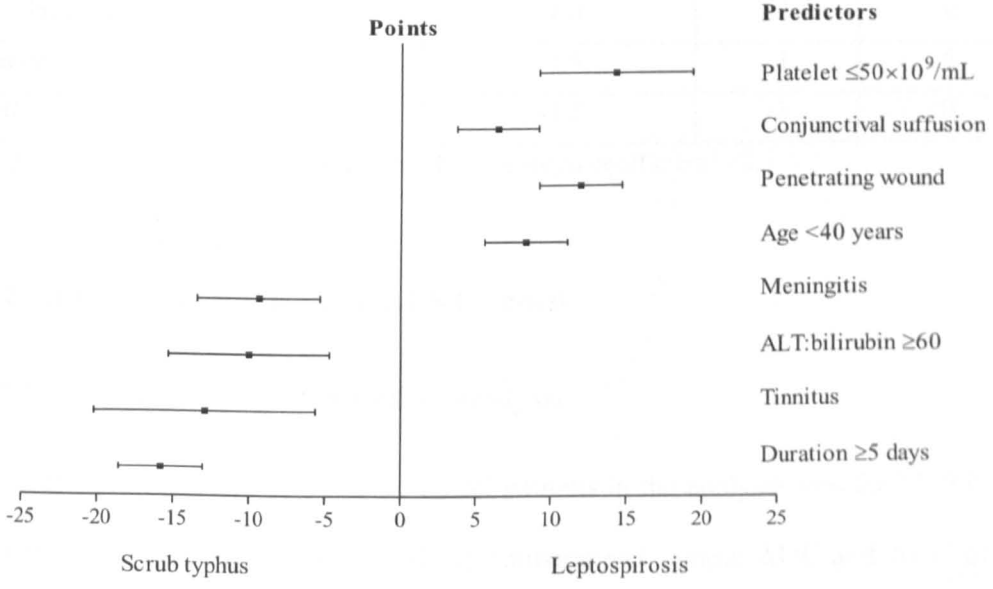

Figure 6.4 Predictors of leptospirosis and scrub typhus among patients with acute febrile illness

For simplicity of the calculation the coefficient for each variable was rounded to the nearest positive or negative .5 or .0 value ranging from -3 to 3 (**Table 6.6**), then the cumulative values were rounded to the closest single digit.

Each variable will give either 0 or the score as appears in the **Table 6.6**. The final score is the summation of 9 marks derived from all variables. The highest and lowest possible values for LEST score are 8 and -10, respectively.

Table 6.6 Leptospirosis-scrub typhus (LEST) score for patients with acute febrile illness with suspected leptospirosis or scrub typhus infection

Predictors	Adjusted coefficient	Final Score§	
		Presence	Absence
Platelet $\leq 50\times10^9$/mL	1.5	3	0
Penetrating wound	1.0	2	0
Age <40 years	1.0	2	0
Conjunctival suffusion	0.5	1	0
Meninigitis	-1.0	-2	0
ALT: direct bilirubin ratio ≥60	-1.0	-2	0
Duration of illness ≥5days	-1.5	-3	0
Tinnitus	-1.5	-3	0

§Final score for each variable was derived by 'Adjusted coefficient' ×2.

6.5.2 Internal validation of LEST Score

6.5.2.1 Bootstrap resampling analysis

The AUC for the LEST score for all patients in the analyses was 0.827 (95%CI: 0.788-0.865). The mean difference of the bootstrapped sample AUC and AUC of the original data using the bootstrapped score following a 1000 bootstrapped resampling simulation was 0.013 (95%CI: 0.012-0.014). The AUC for the LEST score was 0.814 (95%CI: 0.774-0.851) after applying the bootstrapped correction.

6.5.2.2 Predictive value of LEST Score

The predictive ability of the LEST Score is shown in **Figure 6.5**. The optimum cut-off was ≥0 (sensitivity 74.0% and specificity 74.4%) for leptospirosis and <0

(sensitivity 74.4% and specificity 74.0%) for scrub typhus. The score ≥0 correctly identified 166/218 (76.2%) patients as having leptospirosis, and a score <0 correctly identified 148/204 (72.6%) as having scrub typhus.

For higher specificity, the score ≥2 (sensitivity 48.2% and specificity 92.5%) for leptospirosis, score <-2 (sensitivity 47.0% and specificity 91.9%) for scrub typhus, and score -2 to 1 for undetermined diagnoses were used as the cut-off points. With these high specificity cut-off scores, 188/422 (44.6%) of patients fell into the undetermined diagnosis, 107/122 (87.7%) were correctly diagnosed as having leptospirosis, and 94/112 (83.9%) patients were correctly diagnosed as having scrub typhus.

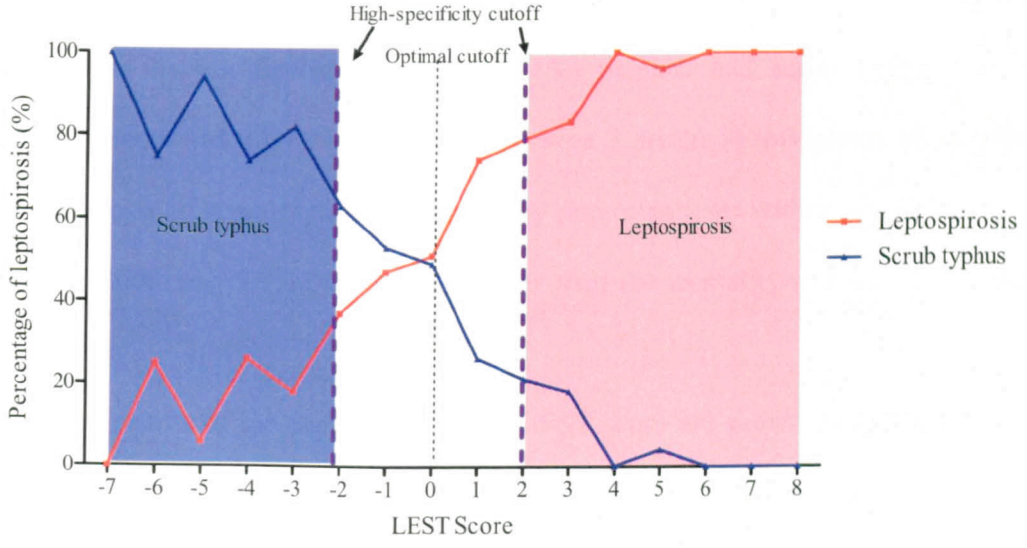

Figure 6.5 Predictive ability of the Leptospirosis-scrub typhus (LEST) score for diagnosing patients with suspected leptospirosis and scrub typhus

6.5.2.3 Co-infection and LEST Score

When the LEST score was calculated for patients with co-infection, 15.8%, 40.0% and 44.2% were identified as scrub typhus, leptospirosis and undetermined diagnoses, respectively. A summary of the diagnosis reached based on the LEST score are shown in **Table 6.7**.

Table 6.7 LEST score diagnoses for all patients, including mixed infections

LEST score category	Patient diagnosis, No.(%)			Total
	Scrub typhus	Mixed infection	Leptospirosis	
Scrub typhus by LEST	94 (47.0)	15 (15.8)	18 (8.0)	127 (24.5)
Undetermined by LEST	91 (45.5)	42 (44.2)	99 (44.2)	232 (44.7)
Leptospirosis by LEST	15 (7.5)	38 (40.0)	107 (47.8)	160 (30.8)
Total	200	95	224	519

6.5.2.4 Further evaluation of the LEST Score

A second database of patients with confirmed leptospirosis and/or scrub typhus admitted to Udon Thani Regional Hospital during July 2003 and December 2006 was used to validate LEST score. There were 160 patients for whom complete data were available for evaluation. The median (IQR) age of patients was 39.5 (28.5-51.0) years old, and there were 117 (73.1%) male patients. Of these, 88 (55.0%) patients were diagnosed as having leptospirosis, 53 (33.1%) patients had scrub typhus, and 19 (11.9%) patients had mixed infection. There were 3 deaths in this group, all of whom had a diagnosis of scrub typhus. The mortality rate of patients with scrub typhus in the year 2003-2006 was 5.7%, which was higher than the mortality rate during the main study period.

The results of the application of the LEST score are shown in **Table 6.8**. With the high specificity cut-off scores, 85/160 (53.1%) of patients fell into the undetermined diagnostic group, 24/29 (87.8%) were correctly diagnosed as having leptospirosis, and 29/36 (80.6%) patients were correctly diagnosed as having scrub typhus.

Table 6.8 LEST score diagnoses among patients with leptospirosis and/or scrub typhus in Udon Thani Regional Hospital, 2003-2006

LEST score category	Patient diagnosis, No.(%)			Total
	Scrub typhus	Mixed infection	Leptospirosis	
Scrub typhus by LEST	29 (54.7)	5 (26.3)	7 (8.0)	41 (25.6)
Undetermined by LEST	19 (35.9)	9 (47.4)	57 (64.8)	85 (53.1)
Leptospirosis by LEST	5 (9.4)	5 (26.3)	24 (27.3)	34 (21.3)
Total	53	19	76	160

6.7 Discussion

The demographic data for patients with leptospirosis was significantly different from patients with scrub typhus for many of the parameters evaluated, especially when the patients with mixed infection were excluded from the analysis. The majority of patients in the mixed infection group had a pattern of clinical presentation that was similar to leptospirosis mono-infection or in-between the two diseases. Some manifestations reflecting the severity of disease, for example, tachypnoea, dyspnoea, subconjunctival haemorrhage, conjunctival suffusion, oliguria and, bleeding diathesis were found in a higher proportion in patients with mixed infection. This may indirectly indicate the severity of mixed infection. Jaundice was noted in nearly half of patients with mixed infection, which is higher than that for patients with either scrub typhus (16%) or leptospirosis (30%). The histopathological changes in the livers of patients with co-infection are unknown. The degree of renal impairment or renal failure was also higher in patients with mixed infections than in patients with either infection alone. The additive effects of dual infections have never been reported previously.

A previous study performed in northeast Thailand reported that patients with mixed infections had significantly higher platelets counts and significantly lower bilirubin and creatinine concentrations than individuals with leptospirosis alone.[305] This included a small number of selected patients (9 mixed infections and 13 leptospirosis patients) and is unlikely to be as statistically robust as the study described here.

When analyses were performed in the two mono-infection groups, the clinical manifestations of leptospirosis were clearly more severe than those of scrub typhus, in terms of APACHE II and SOFA scores. Surprisingly, the mortality was not different between the two diseases. Furthermore, the fever clearance time and length of hospital stay were significantly longer in patients with scrub typhus, despite the fact that most

patients received appropriate antibiotic treatment (85% of scrub typhus patients received oral doxycycline) (data not shown). This raises the possibility that the organism is relatively resistant to the effects of antibiotics, for which there are numerous possible explanations including relative drug resistance, and drug penetration into sites of infection. The median fever clearance time for leptospirosis was around one day.

The presence of atypical lymphocytes found in patients with both leptospirosis and scrub typhus, a more prominent finding in the scrub typhus group, was unexpected and previously unreported. Atypical lymphocytes are often associated with viral infection but this is not absolute. Rickettsioses were once through to be caused by a virus, and so it is possible that this has been observed previously. However, this is the first report of the presence of atypical lymphocytes in patients with leptospirosis.

This study identified clinical features that were significantly different for the two diseases, and this was utilised during the development of a clinical prediction score which was termed as the LEST score. For the purposes of simplicity, continuous data were converted to binomial variables using clinically relevant cut-offs. All of the statistically significant variables were considered. The presence of an eschar could not be used in the LEST score since it was specific for scrub typhus, and cannot present in patients with leptospirosis mono-infection. All eight variables in the final logistic regression model were clearly distinct between the two diseases.

This is the first predictive score developed for the differentiation of two infectious diseases. The target groups for the LEST score are those patients presenting with an acute febrile illness in patients whom either scrub typhus or leptospirosis is suspected. This serve a different purpose to the WHO checklist developed to assess the likelihood of a diagnosis of leptospirosis in humans.[92] When the LEST score was applied to those patients who were judged by the WHO checklist to have leptospirosis, 91% were correctly identified by LEST score, comparing to 86% when used in all

patients suspected leptospirosis. The percentage of patients correctly diagnosed as having scrub typhus using the LEST score was not much changed following the exclusion of patients considered to be non-leptospirosis by the application of the WHO checklist (87% *vs* 86% in all patients) (data not shown). The WHO checklist was not included in the WHO guidance for diagnosis, surveillance and control of leptospirosis revised in 2003.[4]

Evaluation of the LEST score in a second, independent dataset of patients with confirmed leptospirosis and scrub typhus conducted a few years after the initial study showed that the specificity for the diagnosis of scrub typhus had dropped by 10%. One explanation for this may relate to the more severe manifestations of patients with scrub typhus (mortality 5.7% in the new database compared to 3.3% in the original study).

The LEST score represents an easy and rapid bedside test where specific diagnostic tests are not available. Most hospitals in Thailand are able to perform liver function tests including bilirubin, and ALT and complete blood count including platelet count, and these tests are routinely done in most patients. The specificity of the LEST score is higher than the specificity of the latex agglutination test (LA) for leptospirosis[86] and the ELISA test for scrub typhus (84%).[160] The LEST score is highly discriminative in cases where the calculated LEST score is ≥ 4 or ≤ -4, which are suggestive of leptospirosis and scrub typhus, respectively. Antibiotics with activity against both diseases should be prescribed for patients with a LEST score ranging from -2 to 1, which represents the range associated with an undetermined diagnosis.

An important drawback of the LEST score is that it was developed using patients with mono-infection, and is not applicable for patients with mixed infection. The problem remains, therefore, as to how to identify patients with mixed infection. When this score was applied to the mixed infection group, the majority of patients were classified as either undetermined or leptospirosis. Further validation and improvement

of the LEST score is needed for the mixed-infection group. Further validation is also required in other geographic areas where both diseases are endemic. The predictors included in the algorithm are generally available, and include clinical features together with 3 laboratory values (platelet count, bilirubin and alanine aminotransferase).

6.8 Chapter summary

The presentation of leptospirosis and scrub typhus are quite similar, but many significant differences have been identified here between the two diseases. Patients with mixed leptospirosis and scrub typhus infections had features that were similar to leptospirosis for some manifestations but were in-between the two diseases for the others. Patients with leptospirosis were more severely ill than patients with scrub typhus in terms of higher bilirubin, creatinine levels, and bleeding complication, lower platelets concentration, and higher SOFA scores.

The LEST score was developed for the prediction of leptospirosis and scrub typhus. Eight parameters were included: age <40 years, duration of illness ≥5 days, the presence of a penetrating wound, conjunctival suffusion, tinnitus, meningism, platelet concentration $\leq 50 \times 10^9$/mL, ALT:bilirubin ratio ≥60. The LEST score had a greater than 80% specificity for the diagnosis of both leptospirosis and scrub typhus. Further validation is required in clinical practice and in other geographic regions where both diseases are concurrently endemic.

Chapter VII: Concluding comments

This work arose from two important questions frequently asked by doctors working in northeast Thailand. First, what are the common causes of febrile illness in the region? Second, faced with a patient with a febrile illness associated with fever, myalgia and headache, is it possible to differentiate between leptospirosis and scrub typhus at the bedside in the absence of specific diagnostic tests? In the year 2000 when the sustained outbreak in northeast Thailand was at its peak, most patients presenting with an acute febrile illness were diagnosed and treated for leptospirosis because physicians feared the fatal complications associated with this condition. It was a surprise to find here that so many patients presenting during this period had scrub typhus with or without concomitant leptospirosis. This study has shown that both diagnoses were common in study patients, and also indicated that mixed infections are probably common.

In uncomplicated leptospirosis or scrub typhus, the treatment of choice for both diseases is a seven day course of oral doxycycline, so an incorrect diagnosis of scrub typhus in patient with leptospirosis, or vice versa does not lead to the use of inappropriate treatment. For patients with severe clinical manifestations and suspected leptospirosis, the antibiotic treatment choices are intravenous penicillin, cefotaxime or ceftriaxone, drugs that are not active against *O. tsutsugamushi*. The wrong diagnosis in these cases would be associated with a delay in appropriate antibiotic treatment and possibly a fatal outcome. Thus, the findings of this study are likely to impact most on patients with severe disease, although the majority of fatal cases were excluded from the analysis due to a lack of diagnosis confirmation in this group.

The diagnosis in nearly half of all study patients with febrile illness remains unknown. Although a proportion of patients died before a full battery of serological

tests could be performed, this probably may have accounted for a relatively small proportion of unknown diagnoses. The more likely explanation is that the range of diagnostic tests undertaken was limited. This was a function of two factors. One of the major objectives of the study was to determine the common causes of acute febrile illness. Based on clinical features observed before the start of the study, scrub typhus and leptospirosis were high on the list of possibilities and leptospirosis was considered to be one of the major causes of death from acute undifferentiated febrile illness in this setting. This study has been successful in confirming the proportion of patients admitted who had one or both of these diagnoses. The second factor that led to a limited range of tests being performed was available funds and resources at the time the study was conducted. A great strength of the study, however, is that stored samples can be tested in the future to provide a more complete picture of disease epidemiology. Important infectious diseases that may be prevalent in the area but which remain to be evaluated include influenza and parainfluenza, mycoplasma infections and viral hepatitis.

This study is the first to describe and compare the clinical severity and complications of patients with culture-positive and culture-negative leptospirosis, and between patients with presumptive primary scrub typhus and cases of reinfection. Patients with culture-negative leptospirosis had more severe clinical symptoms and a higher rate of complications compared with the culture-positive group, but the mortality was not different between the two. Complications such as aseptic meningitis, jaundice, renal failure, thrombocytopenia and pulmonary haemorrhage were noted to occur very early in the course of illness in some patients, despite previous descriptions of the biphasic nature of this infection in which many of the severe complications are attributed to the second 'immune' phase of disease. Overall clinical presentations of primary scrub typhus and reinfection were not different, with the exception that patients with reinfection were older and presented to hospital earlier than those with primary

infection. Confusion was more common in reinfection, whereas jaundice, liver enzyme elevations and gastrointestinal bleeding were more common in primary infection.

This study has raised awareness for the possibility of mixed infections. In modern medical practice, physicians are trained to find a single explanation for all of the clinical features of an illness in a given patient, but this study suggests that this is not always the case. Co-infections with leptospirosis and scrub typhus were noted in 30% of patients. This provided the opportunity to undertake an evaluation of the clinical features and outcome from mixed infection. Clinical features and disease severity were more severe in patients with mixed infection. Deaths among this group were associated with a delay in administration of effective antimicrobial treatment or inappropriate antibiotic treatment for scrub typhus. Further studies are required using molecular techniques to confirm the frequency of concurrent infection with two or more pathogens, as opposed to acute infection caused by one pathogen combined with a recent past history of infection with a second pathogen. Intuitively, one would predict that mixed infection would be associated with more severe disease, and further studies are required to examine the pathophysiological basis for the clinical findings.

The mortality rate of leptospirosis and scrub typhus were comparable at 3% and 3.3%, respectively. Pulmonary haemorrhage was the leading cause of death in both groups. Pulmonary haemorrhage in patients with scrub typhus is described in the literature but is usually attributed to leptospirosis in the absence of diagnostic tests. Increased awareness of these findings is required at Udon Thani hospital and more widely in Asia, since the treatment given for severe leptospirosis may be inadequate for severe scrub typhus. The fever clearance time and duration of hospitalisation were longer for patients with scrub typhus despite the less severe clinical manifestations in terms of lower APACHE II and SOFA scores. This may indicate the existence of drug resistance scrub typhus in the study population.

Collection of detailed information on patients with scrub typhus and leptospirosis provided the basis for the development of a predictive algorithm for the differentiation of leptospirosis and scrub typhus, termed here as the LEST score. This represents a simple tool that could be applied in clinical settings where diagnostics are not available. This simple score based on clinical features and routine laboratory tests can be used as a substitute for expensive, low-sensitivity, rapid diagnostic tests. Prospective validation of the LEST score is now required to determine its accuracy in routine practice, and to evaluate its applicability and accuracy in other parts of the world where leptospirosis and scrub typhus are both endemic.

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Appendix A: Clinical record form

Leptospirosis

Lepto No
Admission No
◯ Prospective ◯ Retrospective

HN
XN
AN

House No | Moo | Tumbon
District | Province
Age yr | Sex ◯ Male ◯ Female | Record date
Type of patient ◯ Inpatient ◯ Outpatient only

Admission to hospital date
Admission to effective treatment date

Study 1 | Date | Study No
Study 2 | Date | Study No
Study 3 | Date | Study No

Initial diagnosis

☐ R/O Leptospirosis	☐ Scrub typhus	☐ R/O urinary tract infection
☐ Sepsis/septic shock	☐ Systemic infection	☐ Meningitis
☐ Hepatitis	☐ Miliary tuberculosis	☐ Aseptic meningitis
☐ Pneumonia	☐ Fever with convulsion	☐ Alcoholic cirrhosis
☐ Acute cholecystitis	☐ Acute pancreatitis	☐ Nephrotic syndrome
☐ Ascending cholangitis	☐ Eosinophilic meningitis	☐ Upper GI haemorrhage
☐ Acute febrile illness	☐ Acute pyelonephritis	☐ R/O Dengue Haemorrhagic Fev

Initial diagnosis by ◯ Clinical suspected only ◯ Serology ◯ Culture
Discharge date
Discharge status

◯ Improved/with approval	◯ Not improved/escape
◯ Improved/transfer	◯ Not improved/transfer
◯ Not improved/against advice	◯ Death/certain death

Occupational and behavioural at risk

Occupation

☐ Excavator	☐ Farmer:Pig	☐ Livestock	☐ Trader
☐ Farmer:Rice	☐ Fisherman	☐ Medical personel	☐ Worker
☐ Farmer:Non rice	☐ Government officer	☐ Student	☐ Other...

Housing

Floodable/Wet area ◯ Yes ◯ No
Geographic ◯ plain ◯ forest/hills ◯ Other...
Rat ◯ Never ◯ Rarely ◯ Occasionally ◯ Often
Animal stable nearby ◯ Yes ◯ No
Rice field nearby ◯ Yes ◯ No — Sugar cane farm nearby ◯ Yes ◯ No
Fruit garden nearby ◯ Yes ◯ No — Maize farm nearby ◯ Yes ◯ No
Peanut/Soybean farm nearby ◯ Yes ◯ No — Tapioca farm nearby ◯ Yes ◯ No
Reservoir nearby ◯ Yes ◯ No — Can animals access to? ◯ Yes ◯ No

Work area

Floodable/Wet area ◯ Yes ◯ No
Geographic ◯ plain ◯ forest/hills ◯ Other...
Rat ◯ Never ◯ Rarely ◯ Occasionally ◯ Often
Animal farm ◯ Yes ◯ No
Rice field ◯ Yes ◯ No — Sugar cane farm ◯ Yes ◯ No
Fruit garden ◯ Yes ◯ No — Maize farm ◯ Yes ◯ No
Peanut/Soybean farm ◯ Yes ◯ No — Tapioca farm ◯ Yes ◯ No
Reservoir nearby ◯ Yes ◯ No — Can animals access to? ◯ Yes ◯ No

Domesticating or contacting animals within 30 days of onset of symptoms

Dog ◯ Yes ◯ No — Monkey ◯ Yes ◯ No
Cat ◯ Yes ◯ No — Rabbit ◯ Yes ◯ No
Pig ◯ Yes ◯ No — Gibbon ◯ Yes ◯ No
Cow ◯ Yes ◯ No — Horse ◯ Yes ◯ No
Water buffalo ◯ Yes ◯ No — Frog ◯ Yes ◯ No
Mouse/rat ◯ Yes ◯ No — Gecko ◯ Yes ◯ No
Squirrel ◯ Yes ◯ No — Grasshopper ◯ Yes ◯ No
Bird ◯ Yes ◯ No — Other animals ◯ Yes ◯ No Specify_____
Chicken ◯ Yes ◯ No

If "Yes", please give detail:_____

2

Present history

Lepto No

Admission No

Onset of symptom

Month of onset of symptom

Admission mode ○ Not recorded ○ From home ○ Referred from other hospital

If referred:

Other hospital diagnosis

- ☐ Not recorded/from home
- ☐ R/O Leptospirosis
- ☐ Scrub typhus
- ☐ Systemic infection
- ☐ Sepsis/septic shock
- ☐ Hepatitis
- ☐ Pneumonia
- ☐ Acute pyelonephritis
- ☐ Biliary tract infection
- ☐ Acute febrile illness
- ☐ Thalassaemia with fever
- ☐ Upper GI bleeding
- ☐ Encephalitis
- ☐ Viral infection
- ☐ Acute cholecystitis
- ☐ Fever with jaundice
- ☐ Acute haemorrhagic fever
- ☐ R/O CNS infection
- ☐ Meningitis
- ☐ Urinary tract infection
- ☐ Other...

Duration of other hosp admission ☐ day

Treatment from other hospital

	Drug/Dose	Duration
1		
2		
3		
4		

Premorbid medication

	Drug/Dose
1	
2	
3	

Present illness

"0"= No, "Other..."please state duration in day

Fever	○ Not recorded ○ 0 ○ Other...	Cyanosis	○ Not recorded ○ 0 ○ Other...
Chills	○ Not recorded ○ 0 ○ Other...	Cough	○ Not recorded ○ 0 ○ Other...
Malaise	○ Not recorded ○ 0 ○ Other...	Sputum	○ Not recorded ○ 0 ○ Other...
Myalgia	○ Not recorded ○ 0 ○ Other...	Tachypnoea	○ Not recorded ○ 0 ○ Other...
Tiredness	○ Not recorded ○ 0 ○ Other...	Dyspnoea	○ Not recorded ○ 0 ○ Other...
Pallor	○ Not recorded ○ 0 ○ Other...	Orthopnoea	○ Not recorded ○ 0 ○ Other...
Oedema	○ Not recorded ○ 0 ○ Other...	Palpitation	○ Not recorded ○ 0 ○ Other...
Arthralgia	○ Not recorded ○ 0 ○ Other...	Chest pain	○ Not recorded ○ 0 ○ Other...
Swollen joint	○ Not recorded ○ 0 ○ Other...	Sore throat	○ Not recorded ○ 0 ○ Other...
		Hoarseness	○ Not recorded ○ 0 ○ Other...

Jaundice	○ Not recorded ○ 0 ○ Other...	Neck pain	○ Not recorded ○ 0 ○ Other...
Weight loss	○ Not recorded ○ 0 ○ Other...	Back pain	○ Not recorded ○ 0 ○ Other...
Anorexia	○ Not recorded ○ 0 ○ Other...	Loin pain	○ Not recorded ○ 0 ○ Other...
Nausea	○ Not recorded ○ 0 ○ Other...	Cloudy/turbid urine	○ Not recorded ○ 0 ○ Other...
Vomiting	○ Not recorded ○ 0 ○ Other...	Polyuria	○ Not recorded ○ 0 ○ Other...
Dysphagia	○ Not recorded ○ 0 ○ Other...	Oliguria/anuria	○ Not recorded ○ 0 ○ Other...
Abdominal pain	○ Not recorded ○ 0 ○ Other...	Urinary retention	○ Not recorded ○ 0 ○ Other...
	☐ Not recorded ☐ Paraumbilicus ☐ RLQ ☐ Generalized ☐ Suprapubic ☐ LUQ ☐ Epigastrium ☐ RUQ ☐ LLQ	Frequently urination	○ Not recorded ○ 0 ○ Other...
Constipation	○ Not recorded ○ 0 ○ Other...	Dysuria	○ Not recorded ○ 0 ○ Other...
Diarrhoea	○ Not recorded ○ 0 ○ Other...	Dark urine	○ Not recorded ○ 0 ○ Other...

Vaginal discharge ○ Not recorded ○ 0 ○ Other...

Dizziness	○ Not recorded ○ 0 ○ Other...	Behavioural change	☐ Not recorded ☐ Dementia ☐ No evidence ☐ Psychosis ☐ Mood lability ☐ Depression ☐ Irritability ☐ Mania ☐ Irrational ☐ Other...
Syncope	○ Not recorded ○ 0 ○ Other...		
Headache	○ Not recorded ○ 0 ○ Other...		
	○ Not recorded ○ Mild ○ Moderate ○ Severe		
	○ Not recorded ○ Sudden ○ Insidious		
	○ Not recorded ○ Intermittent ○ Persistent		
	○ Not recorded ○ Dull aching ○ Throbbing ○ Bursting ○ Other...		
Deteriolation of consciousness	○ Not recorded ○ 0 ○ Other...	Weakness	○ Not recorded ○ 0 ○ Other...
Confusion	○ Not recorded ○ 0 ○ Other...	Hearing impairment	○ Not recorded ○ 0 ○ Other...
Convulsions	○ Not recorded ○ 0 ○ Other...	Facial palsy	○ Not recorded ○ 0 ○ Other...
Twitching	○ Not recorded ○ 0 ○ Other...	Amnaesia	○ Not recorded ○ 0 ○ Other...
Spasticity	○ Not recorded ○ 0 ○ Other...	Hallucination	○ Not recorded ○ 0 ○ Other...
		Other NS abnormality	☐ None ☐ Numbness ☐ Aphasia ☐ Other...

		Conjunctival suffusion	○ Not recorded ○ 0 ○ Other...
		Photophobia	○ Not recorded ○ 0 ○ Other...
Wound/Ulcer	○ Not recorded ○ 0 ○ Other...	Eye redness	○ Not recorded ○ 0 ○ Other...
Eschar	○ Not recorded ○ 0 ○ Other...	Ear pain	○ Not recorded ○ 0 ○ Other...
Skin rash	○ Not recorded ○ 0 ○ Other...	Ear discharge	○ Not recorded ○ 0 ○ Other...
Itching	○ Not recorded ○ 0 ○ Other...	Nasal discharge	○ Not recorded ○ 0 ○ Other...
Cellulitis	○ Not recorded ○ 0 ○ Other...		
Abscess	○ Not recorded ○ 0 ○ Other...		
Subcutaneous mass	○ Not recorded ○ 0 ○ Other...		

Petechiae/Bruise	○ Not recorded ○ 0 ○ Other...
Epistaxis	○ Not recorded ○ 0 ○ Other...
Haemoptysis	○ Not recorded ○ 0 ○ Other...
Bleeding per gum	○ Not recorded ○ 0 ○ Other...
Haematemesis	○ Not recorded ○ 0 ○ Other...
Melaena	○ Not recorded ○ 0 ○ Other...
Haematochaesia	○ Not recorded ○ 0 ○ Other...
Haematuria	○ Not recorded ○ 0 ○ Other...
Bleeding per vagina	○ Not recorded ○ 0 ○ Other...

"0"= No, "Other..."please state duration in day

Lepto No

Date | Physical examination | Weight kg

Temp C | RR /min | SBP mmHg | DBP mmHg | JVP cm

General condition: Unknown / Good / Fair / Moderate / Serious / Critical

Peripheral perfusion: Not recorded / Good / Fair / Poor

Nutrition: Not recorded / Good / Fair / Poor

Dehydration: Unknown / None / Mild / Moderate / Severe

Oedema: Not recorded / No / Generalized / Pretibial / Lid / Face / Feet

Anaemia: Unknown / None / Mild / Moderate / Severe

Cyanosis: Unknown / None / Peripheral / Central

Jaundice: Unknown / None / Mild / Moderate / Severe

Skin and subcutaneous tissue

Spider nevi: Not recorded / No / Yes

Palmar erythema: Not recorded / No / Yes

Cellulitis: Not recorded / No / Yes

Wound/ulcer: Not recorded / No / Yes — Not recorded / Generalized / Legs / Arms / Trunk / Foot / Hand

Eschar lesion: Not recorded / No / Yes — Axillary area / Groin area / Paraumbilical / Back / Scrotum / Chest / O

Abscess: Not recorded / No / Yes — Not recorded / Generalized / Legs / Arms / Trunk / Foot / Hand

Subcutaneous nodule: Not recorded / No / Yes — Not recorded / Generalized / Pretibial / Legs / Arms / Trunk

Skin rash: Not recorded / No evidence / Urticaria / Macula / Mobilliform / Confluent / Papular — Not recorded / Generalized / Legs / Arms / Trunk / Foot / Hand

HEENT

Conjunctival suffusion: Not recorded / No evidence / Left / Right / Both

Conjunctivitis: Not recorded / No evidence / Left / Right / Both

Subconjunctival haemorrhage: Not recorded / No evidence / Left / Right / Both

Pupil size Lt mm: Not recorded / Normal / Sluggishly react / Not react

Pupil size Rt mm: Not recorded / Normal / Sluggishly react / Not react

Uveitis: Not recorded / No evidence / Left / Right / Both

Ear: Not recorded / Normal / Abnormal

Parotid: Not recorded / Normal / Abnormal

Rhinitis: Not recorded / No evidence / Left / Right / Both

Epistaxis: Not recorded / No evidence / Left / Right / Both

Pharynx: Not recorded / Normal / Abnormal

Lymph node: Not recorded / Normal / Enlarged — Not recorded / <1 cm / 1-2 cm / >2 cm

Not recorded / Cervical / Submandibular / Supraclavicular / Postauricular / Axillary / Epitrochial / Inguinal / Femoral / Other...

Consistency: Not recorded / Soft / Cystic / Firm / Stoney hard

Not recorded / Not tender / Tender

Respiratory system: Not recorded / Normal / Abnormal — Abnormal: Any abnormality(ies) listed below is(are) found.

Rhythm: Not recorded / Regular / Rapid shallow / Kaussmal / Chynne Stoke / Irregular

Sputum: Not recorded / None / Mucoid (white) / Purulent (pus/green/yellow) / Bloody

Dyspnoea: Not recorded / No / Yes

Ventilated: Not recorded / No / Yes

Breath sounds: Not recorded / Normal / Bronchial BS / Coarsed BS / Decreased BS — Not recorded / Lt upper / Lt middle / Lt lower / Rt upper / Rt lower / Generalized

Crepitations: Not recorded / No evidence / Fine / Medium / Coarse — Not recorded / Lt upper / Lt middle / Lt lower / Rt upper / Rt lower / Generalized

Rhonchi: Not recorded / No evidence / Occasional / Wheezing — Not recorded / Lt upper / Lt middle / Lt lower / Rt upper / Rt lower / Generalized

Pleural rub: Not recorded / No / Yes

Cardiovascular system: Not recorded / Normal / Abnormal — Abnormal: Any abnormality(ies) listed below is(are) found.

Pulse rate /min Rhythm: Not recorded / Regular / Irregular / Total irregularity(AF)

Apex: Not recorded / 5th ICS,Lt MCL / Lat to Lt MCL / Lt Ant axillary line

Heaving: Not recorded / No / Yes — Not recorded / Apical / RV

Thrill: Not recorded / No / Yes — Not recorded / MV area / PV area / AV area / Upper Rt parasternal / Carotid

Heart sounds: Not recorded / Normal S1,S2 / Loud S2(P2) / Single S1 / Fixed split S1 / Other...

Murmur: Not recorded / No evidence / Mid systolic / Pansystolic / Diastolic — Not recorded / MV area / PV area / AV area / Upper Rt parasternal / Carotid

Pericardial rub: Not recorded / No / Yes

Gastrointestinal system: Not recorded / Normal / Abnormal — Abnormal: Any abnormality(ies) listed below is(are) found.

Abdomen: Not recorded / Flat / Distended / Surgical scar/wound / Other...

Not recorded / Soft / Tensed / Guarding / Rigidity / Other...

Not recorded / Not tender / Mild tender / Moderately tender / Markedly tender / Rebound tenderness / Other... — Not recorded / Generalized / Epigastrium / Paraumbilicus / Suprapubic / RUQ / RLQ / LUQ / LLQ

Liver: Not recorded / Normal / Abnormal — Not recorded / Not enlarged / Just palpable / Other... cm below RCM

Not recorded / Blunt / Sharp

Not recorded / Smooth / Nodular — Not recorded / Soft / Cystic / Firm

Not recorded / Not tender / Tender

Spleen: Not recorded / Normal / Abnormal — Not recorded / Not enlarged / Dullness on percussion / Just palpable / Other... cm below LCM

Not recorded / Soft / Cystic / Firm

Not recorded / Not tender / Tender

KUB system

CVA: Not recorded / Not tender / Tender

Genitalia: Not recorded / Normal / Abnormal

Prostate: Not recorded / Not enlarged / Enlarged

Joints ○ Not recorded ○ Normal ○ Abnormal

Bone ○ Not recorded ○ Normal ○ Abnormal

Nervous system

Level of consciousness ○ Not recorded ○ Cloudy sensorium ○ Unrousable ○ Fully conscious ○ Stuporous but rousable ○ Areflexic

Glassgow coma score Eyes ☐ Verbal ☐ Motor ☐ Total ☐

Stiffneck ○ Not recorded ○ No ○ Yes

Fundi ○ Not recorded ○ Normal ○ Retinopathy ○ Papilloedema ○ Other...

Retinal haemorrhage ○ Not recorded ○ No evidence ○ Left ○ Right ○ Both

Exudate ○ Not recorded ○ No evidence ○ Left ○ Right ○ Both

Cranial nerve abnormalty ○ Not recorded ○ Facial palsy ○ CN VI palsy ○ Numbness ○ No evidence ○ CN III palsy ○ Aphasia ○ Other...

Muscle ○ Not recorded ○ Normal ○ Flaccid(hypotonia) ○ Spastic(hypertonia)

Abnormal movement ☐ Not recorded ☐ Fasciculation ☐ Cogwheel rigidity ☐ Other... ☐ No evidence ☐ Twitching ☐ Flapping tremor

Muscle power ○ Not recorded ○ Normal (Gr V) ○ Gr IV ○ Gr III ○ Gr II ○ Gr I ○ Gr 0

Muscle tenderness ○ Not recorded ○ No ○ Yes **If yes, specify below**

Calves	○ Not recorded ○ No ○ Yes	Neck	○ Not recorded ○ No ○ Yes
Thigh	○ Not recorded ○ No ○ Yes	Shoulder	○ Not recorded ○ No ○ Yes
Buttock	○ Not recorded ○ No ○ Yes	Forearm	○ Not recorded ○ No ○ Yes
Back	○ Not recorded ○ No ○ Yes	Arm	○ Not recorded ○ No ○ Yes

Reflex ○ Not recorded ○ Normal ○ Hypereflexia ○ Hyporeflexia ○ Areflexia

Abnormal reflex ☐ Not recorded ☐ Trousseau's ☐ Palmomental ☐ Ankle clonus ☐ No evidence ☐ Hoffman's ☐ Rooting ☐ Other... ☐ Chvostek's ☐ Tromner's ☐ Grasping

Plantar response ○ Not recorded ○ No response ○ Flexor ○ Extensor ○ Withdrawal

Haematology

Lepto No ☐

Number of admission ☐

	1	2	3	4	5	6	7	8	9	10
Date										
Complete blood count										
Hb										
Hct (PCV)										
WBC										
%Neutrophils										
%Lymphocyte										
%Monocyte										
%Eosinophils										
%Basophils										
%Young WC										
%Band form										
%Atypical Lymph										
Nucleated RC										
Toxic granule										
Platelet smear										
Platelet count										
MCV										
MCH										
MCHC										
Coagulogram										
PT patient/control										
PT ratio										
PTT patient/control										
INR										
Bleeding time										
Fibrinogen										
FDP										
D-dimer										
Direct Coomb's test										
Indirect Coomb's test										
ESR										
LE cells										
G6PD										
Blood group	_____ Rh_____									
Malaria										
Hb typing		%A___ %A2___ %F____ %F____ Other_____								
Inclusion body										
Other										
Other										

Biochemistry

Lepto No
Number of admission

	Normal range	1	2	3	4	5	6	7	8	9	10
Date											
Glucose	70-100										
BUN	8-26										
Creatinine	0.7-1.5										
Sodium	135-155										
Potassium	3.5-5.5										
Chloride	97-109										
HCO3	23-31										
Albumin	3-5										
Bilirubin	2-3										
SGOT	5-40										
SGPT	5-35										
Alk phos	39-117										
Total bili	0.5-1.5										
Direct bili	0.2-0.8										
Gamma GT											
Total chol	0-200										
HDL-chol	35-79										
Triglyceride	0-200										
Calcium	8-11										
Phosphate	2.5-4.5										
Magnesium	1.7-5										
LDH	230-460										
Total CK	0-175										
CK-MB	up to 25										
Troponin T											
Amylase	30-220										
Lactate											

Urinalysis

	1	2	3	4	5
Date					
pH					
Sp gr					
Protein					
Sugar					
WBC					
RBC					
Bilirubin					
Casr					

Other test

Date				
T3				
T4				
Free T4				
TSH				
Cortisol				

Serology

Lepto No_____ No of admission____

	1	2	3	4	5
Date					
Laboratory					
DNA for method					
Anti-HEV Ab					
VDRL					
HBeAg					
Anti-HBe Ab					
Anti-HBc Ab					
Anti-HCV Ab					
Anti-DNA					
ANF					
Rheumatoid factor					
Other					
Other					

Leptospira antibody

Day	1	4	8	15		
Date						
MCAT						
Dipstick						
IFA						
ELISA						
Latex						
Lateral flow						

Scrub typhus antibody

Day	1	4	8	15		
Date						
Dip-S-Ticks						
IFA						

CSF

Date	Time	OP/CP	Volume	Appearance	Prot/Sug	G/S	Indian ink	Crypto Ag	Cell count	N	L	Eo	RC	Culture

Blood gas

Lab														
Date														
Time														
pH														
pCO2														
pO2														
HCO3														
TCO2														
ABE														
SO2														
FiO2														
Resp rate														
TV														
PF/PS														
PEEP														
Mode														

Clinical record from

Microbiology

Lepto No

Number of admission

Specimen	Date	Day*	Lab I=	Lab W	Microscopy	DF	Culture result: No growth	Lepto	Other path	Other micr	Remarks	Sensitivity pattern			
Blood															
Respiratory															
Pus or fluid															
Urine															

* Day 1 = Admission day

Radiology

Plain abdomen, KUB

Date	1	2	3
Normal			
Nephrocalcinosis			
Renal calculi			
Staghorn calculi			
Ureteric calculi			
Vesicular calculi			
Hepatomegaly			
Splenomegaly			
Ileus			
Pancreatic calcification			
Soft tissue mass			
Ascites			
Osteoporosis			
Other abnormality			

Ultrasonography

Investigation / Date			
Normal			
Single hepatic abscess			
Multiple hepatic abscesses			
Probable hepatic abscess			
Hepatomegaly			
Cirrhosis			
Gallstone			
Calcific liver granuloma			
Probable cholangioCA			
Single splenic abscess			
Multiple splenic abscesses			
Splenomegaly			
Calcific splenic granuloma			
Pancreatic calcification			
Pancreatic mass			
Subphrenic abscess			
Soft tissue abscess			
Mesenteric cyst			
Ileus			
Ascites			
Renal/perirenal abscess			
Renal parenchymatous disease			
Hydronephrosis			
Urinary tract calculi			
Polycystic kidney/liver			
Pericardial effusion			
Pleural effusion			
Other			
Other			

Yes or No

Lepto No

Intravenous pyelography

Date	1	2	3
Normal			
Impaired Lt renal function			
Impaired Rt renal function			
Impaired bilat renal function			
Lt UC: non obstructive			
Rt UC: non obstructive			
Bilat UC: non obstructive			
Lt UC: obstructive			
Rt UC: obstructive			
Bilat UC: obstructive			
Other abnormality			

Bone and Joint

Investigation / Date			
Normal			
Osteoporosis/demineralisation			
Osteomyelitis			
Joint effusion			
Soft tissue swelling			
Fracture			
Arthritis			
Abscess with air-fluid level			
Degenerative changes			
Spondylolithesis			
Other			

Brain CT

Date	1	2	3
Normal			
Cerebral infarction			
Intracerebral haemorrhage			
Cerebral atrophy			
Cerebral oedema, swelling			
Abscess			
Cerebritis			
Other abnormality			

Other film or CT scan

Investigation			
Date			
Normal			
Abnormal_____			
Abnormal_____			
Abnormal_____			
Abnormal_____			

Treatment during admission

Lepto No._____
No of admission____

Treatment effective to Leptospirosis

Parenteral treatment

Drugs	Dose	Interval	Start date	Stop date	Duration

Oral treatment

Drugs	Dose	Interval	Start date	Stop date	Duration

Other antibiotics

Drugs	Dose	Interval	Start date	Stop date	Duration

Other treatment

Drugs	Dose	Interval	Start date	Stop date	Duration

Haemodialysis	No	Yes	_____times
FFP	No	Yes	_____ml (____units)
Platelet concentration	No	Yes	_____units
Blood(PRC, WB)	No	Yes	_____units

Drug toxicity

Not recorded	
No evidence	
Nausea	
Retching	
Diarrhoea	
GI bleeding	
Abdominal pain	
Flatulence	
Skin rash	
EM	
SJS	
Itching	
Hepatitis	
Renal failure	
Haematologic disorders	
Numbness	
Palpitation	
Others	

Home medication

Drugs	Dose	Interval

Summary

Complication

Hypotension ☐
Myocarditis ☐
Acute renal failure ☐
Rhabdomyolysis ☐
Lab coagulopathy ☐
subcutaneous bleeding ☐
GI bleeding ☐
Haemoptysis ☐
ulmorary haemorrhage ☐
Respiratory failure ☐
Pulmonary oedema ☐

Jaundice/liver dysfunction ☐
Pancreatitis ☐
Hypoglycaemia ☐
Hyperglycaemia ☐
Anaemia ☐
Thrombocytopaenia ☐
Neutropaenia ☐
Pancytopaenia ☐
Super infection ☐
Vascular occlusion ☐
Uveitis ☐

Lepto No ☐
Admission No ☐
Reactive arthropathy ☐
Convulsions ☐
Polyneuropathy ☐
Aseptic meningitis ☐
Neuritis ☐
Psychosis ☐
Mental disturbance ☐
Persistent headache ☐

State "Yes" or "No"

Summary

Discharge date ☐

Final diagnosis ○ Leptospirosis ○ Acute febrile illness ○ R/O Scrub typhus ○ R/O Dengue Haema ○ R/O Leptospirosis ○ Scrub typhus ○ Murine typhus ○ Viral hepatitis

Leptospirosis ☐ Clinical signs and symptoms only ☐ Positive screening serologic test ☐ High titers of single IFA IgM ☐ Four folds rising of IFA IgM ☐ High titers of single MAT ☐ Four folds rising of MAT titers

ighest temperature ☐ Effective treatment starting hour ☐ Date ☐
Fever clearance ☐ Fever clearance hour ☐ Date ☐

Evaluability ○ Evaluable ○ Switched regimen ○ Exclusion ○ Died within 48 hours ○ Not in drug trial

Acute outcome ○ Alived ○ Died within 48 hours ○ Died ○ Certain death ○ Uncertain

Time to discharge from treatment ☐ day
Time to discharge from admission ☐ day

Follow up ☐

1st Follow up date ☐ Outcome at 1st follow up ○ Not known ○ Well ○ Complication from treatment ○ Complication from disease ○ Undetermined complication

2nd Follow up date ☐ Outcome at 2nd follow up ○ Not known ○ Well ○ Complication from treatment ○ Complication from disease ○ Undetermined complication

3rd Follow up date ☐ Outcome at 3rd follow up ○ Not known ○ Well ○ Complication from treatment ○ Complication from disease ○ Undetermined complication

Clinical record from

Appendix B: Cerebrospinal fluid profiles of 65 patients with leptospirosis and/or scrub typhus

	Patient code	Diagnosis	duration of symptom (day)	Open pressure (mmH_2O)	Close pressure (mmH_2O)	Appearance	Protein (g/dL)	Sugar (mg/dL)	WBC count	% N	% Lym	% Eo
1	13	L	4	13	8	Clear	57	81	1	0	100	0
2	19	S	7	9.5	7.0	Clear	76	51	2	0	100	0
3	20	L	3	25	17	Clear	30	82	0	0	0	0
4	21	S	4	27	24	Clear	30	82	25	0	100	0
5	27	L	8	na	na	Clear	43	51	283	10	85	5
6	37	S	7	17	14.5	Clear	93	45	8	0	100	0
7	46	S	4	33.5	27.5	Clear	117	70	133	0	100	0
8	48	S	3	24	16	Clear	114	60	46	32	68	0
9	49	S	5	22	15	Clear	17	54	1	0	100	0
10	50	S	2	25	22	Turbid	261	54	190	5	95	0
11	53	S	5	9	7	Clear	19	80	2	0	100	0
12	55	S	12	18	14.5	Clear	na	na	13	2	98	0
13	56	S	12	19	16	Clear	72	61	7	0	100	0
14	62	S	9	35	31	Clear	9	69	9	0	100	0
15	63	S	9	25	23.5	Clear	334	57	190	0	100	0
16	73	S	7	16.5	15.5	Clear	68	69	2	0	100	0
17	82	L	3	20	18	Clear	53	18	6	0	100	0
18	99	S	11	na	na	Turbid	120	39	850	3	47	50
19	100	S	4	na	na	Clear	66	37	12	10	90	0
20	101	L	5	9	7	Clear	18	63	1	0	100	0
21	102	S	10	12.5	11	Clear	57	49	1	0	100	0
22	116	L	1	14	12.5	Clear	15	74	0	0	0	0
23	119	S	6	19.5	15	Clear	68	55	36	5	95	0
24	120	L	6	16	12.5	Clear	55	53	55	8	92	0
25	172	L	6	na	na	Clear	33	55	0	0	0	0
26	250	S	4	na	na	Clear	75	67	10	0	100	0
27	254	S	9	na	na	Clear	42	74	1	0	100	0
28	259	S	9	na	na	Clear	365	61	32	0	100	0
29	263	L	3	na	na	Clear	46	58	12	0	100	0
30	277	L	3	na	na	na	na	na	na	na	na	na
31	440	S	5	na	na na	Clear	119	60	159	10	90	0
32	442	L, S	1	na	na	Clear	46	50	2	0	100	0
33	479	L, S	7	na	na	Clear	146	69	125	5	95	0

na: not available, L: leptospirosis, S: scrub typhus, M: murine typhus, N: neutrophils, Lym: lymphocytes, Eo: eosinophils

Appendix B (cont.) Cerebrospinal fluid profiles of 65 patients with leptospirosis and/or scrub typhus

	Patient code	Diagnosis	duration of symptom (day)	Open pressure (mmH_2O)	Close pressure (mmH_2O)	Appearance	Protein (g/dL)	Sugar (mg/dL)	WBC count	% N	% Lym	% Eo
34	499	S	1	16	13	Clear	22	65	0	0	0	0
35	537	S	3	20.5	15	Clear	146	49	12	90	30	0
36	586	S	15	na	na	Clear	81	63	17	30	70	0
37	595	S	3	na	na	Clear	101	57	39	50	50	0
38	650	S	5	na	na	Clear	121	66	45	0	100	0
39	664	S	3	na	na	na	na	na	na	na	na	na
40	665	S	12	27	22	Clear	146	51	85	2	98	0
41	676	S	3	na	na	Clear	159	63	496	10	90	0
42	688	L, S	7	na	na	Clear	134	43	78	1	99	0
43	754	L	6	na	na	Turbid	12	54	42	70	30	0
44	756	L	3	na	na	Clear	174	59	295	2	98	0
45	768	S	6	na	na	Clear	27	53	0	0	0	0
46	789	L, S	9	na	na	na	na	na	na	na	na	na
47	796	S	4	43	30	Clear	103	46	58	4	96	0
48	831	S	5	na	na	Clear	89	67	20	15	85	0
49	863	S	6	na	na	Clear	129	37	10	0	100	0
50	870	S	6	na	na	Turbid	101	60	11	0	100	0
51	871	S	3	na	na	Turbid	51	48	430	0	100	0
52	904	S	2	na	na	Clear	na	na	0	0	0	0
53	907	S	3	na	na	Clear	16	51	1	0	0	0
54	927	L, S	8	na	na	na	na	na	na	na	na	na
55	933	S	3	na	na	Clear	124	53	38	10	90	0
56	1007	L	3	na	na	na	na	na	na	na	na	na
57	1088	L	6	na	na	na	na	na	na	na	na	na
58	1118	L, M	2	na	na	Clear	10	78	1	0	100	0
59	1156	S	6	na	na	na	na	na	na	na	na	na
60	1168	L	14	na	na	na	na	na	na	na	na	na
61	1171	L	5	na	na	Clear	43	64	3	0	100	0
62	1174	S	4	na	na	Clear	295	36	126	15	82	3
63	1190	L	3	16	10	Clear	15	60	0	0	0	0
64	1207	L	5	na	na	Clear	12	57	0	0	0	0
65	1227	L	4	na	na	Clear	42	55	7	0	100	0

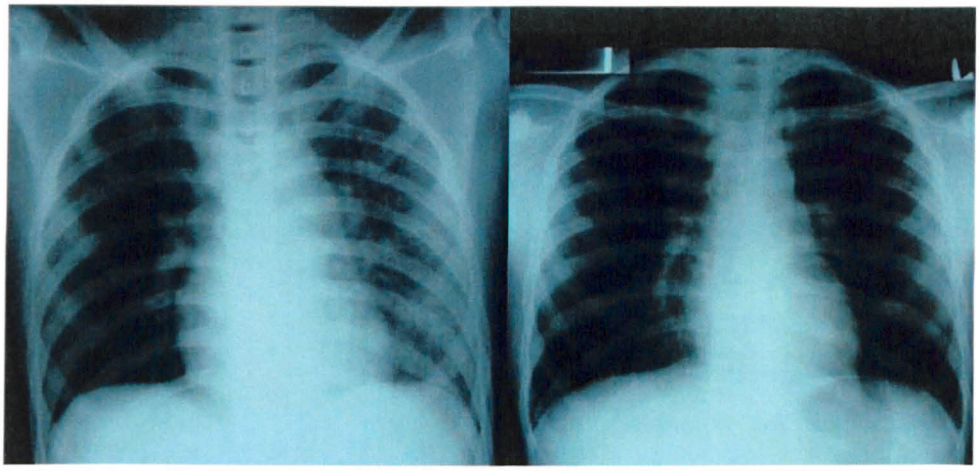
na: not available, L: leptospirosis, S: scrub typhus, M: murine typhus, N: neutrophils, Lym: lymphocytes, Eo: eosinophils

Appendix C: Clinical manifestations of 10 patients who died from leptospirosis and/or scrub typhus

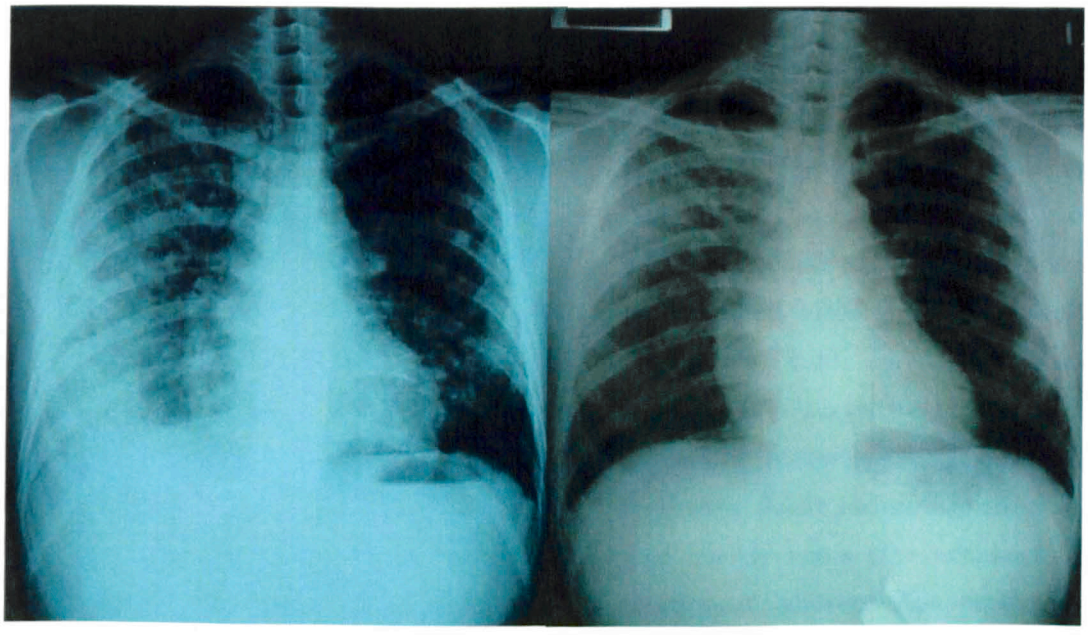
Code	Age, gender	Diagnosis	duration of illness	Clinical manifestations	Treatment
41	57, male	S	13 days	BP 120/80 mmHg (on inotropic), renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	cefotaxime, gentamicin
244	31, female	S	9 days	BP 140/90 mmHg (on inotropic), renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	cefotaxime, metronidazole
254	65, male	S	8 days	BP 135/69 mmHg, encephalopathy, meningitis, convulsion, thrombocytopenia	penicillin, doxycycline
351	31, male	L, S	4 days	BP 110/70 mmHg, renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	penicillin
453	71, male	L	4 days	BP 80/50 mmHg, renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	penicillin
456	52, male	S	7 days	BP 110/70 mmHg (on inotropic), renal failure, jaundice, thrombocytopenia	cefotaxime
518	59, female	L	4 days	BP 90/50 mmHg (on inotropic), renal failure, thrombocytopenia, pulmonary haemorrhage	penicillin, gentamicin, ciprofloxacin
551	22, male	L	2 days	BP 89/31 mmHg (on inotropic), thrombocytopenia, pulmonary haemorrhage	penicillin, cefotaxime
563	33, male	L	3 days	BP 88/58 mmHg (on inotropic), renal failure, thrombocytopenia	penicillin, cefotaxime, meropenem
643	38, male	L	3 days	BP 60/30 mmHg (on inotropic), renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	penicillin, doxycycline, ceftazidime
673	29, male	L, S	5 days	BP 100/70 mmHg, renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	doxycycline, cefotaxime, gentamicin
681	59, male	S	4 days	BP 80/50 mmHg (on inotropic), renal failure, jaundice, pulmonary haemorrhage	ceftazidime
764	29, male	S	3 days	BP 120/80 mmHg (on inotropic), convulsion, encephalopathy,	cefotaxime
773	55, female	L	2 days	BP 78/48 mmHg (on inotropic)	penicillin, ceftriaxone, amikacin
880	53, female	S	11 days	BP 91/52 mmHg (on inotropic)	cefotaxime, doxycycline, ceftazidime
1035	55, male	L	8 days	BP 100/60 mmHg, renal failure, pulmonary haemorrhage	cefotaxime, doxycycline, ceftazidime
1126	44, female	L	3 days	BP 92/60 mmHg (on inotropic), renal failure, jaundice, thrombocytopenia, pulmonary haemorrhage	penicillin, cefotaxime
1176	52, male	S	2 days	BP 60/40 mmHg, renal failure	penicillin, cefotaxime, ceftazidime

Fatal cases

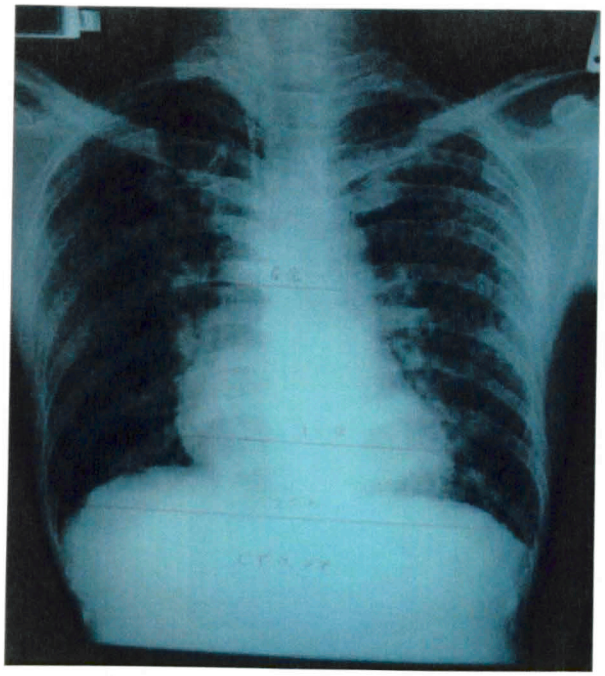
Appendix D: Chest radiographs of patients with leptospirosis and pulmonary haemorrhage



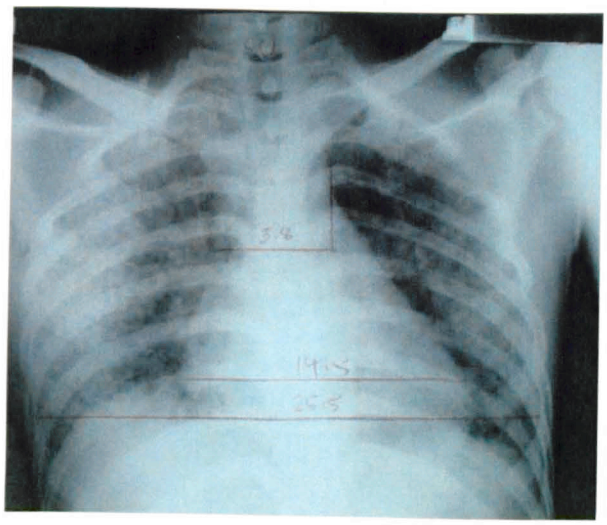
Patient L17: 15 year old boy with fever for 2 days, epistaxis and haemoptysis developed just before admission. Left: chest radiograph performed on admission, bilateral diffused nodular infiltrations, prominently on the left lung. Right: 5 days after the first film, resolved of the infiltrations.



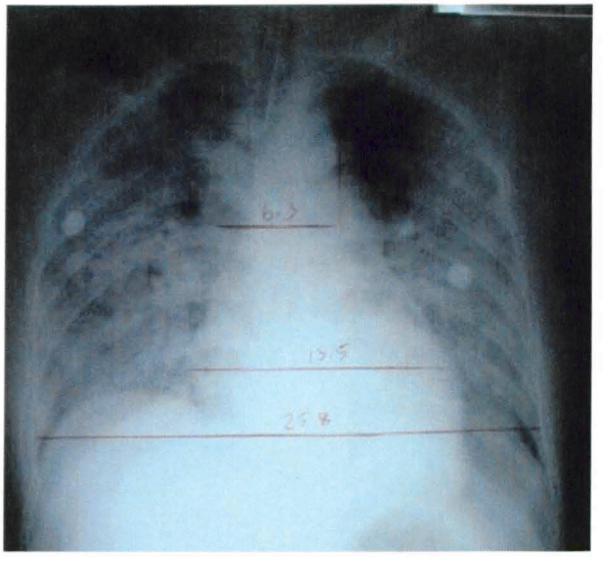
Patient L71: 22 year old man with fever for 4 days, blood-streaked sputum developed 3 days before admission. Left: chest radiograph performed on admission, bilateral diffused nodular infiltrations, prominently on the right lung. Right: 3 days after the first film, improving of the infiltrations.



Patient 378: 32 year old man with fever and jaundice for 5 days, blood-streaked sputum and melaena developed 2 days before admission. Creatinine level was 5.6mg/dL, platelet was 13×10^9/mL, and total bilirubin level was 11.1mg/dL on admission and was raised to 40.1mg/dL 5 days thereafter. Chest radiograph performed on admission, bilateral diffused nodular infiltrations with cardiomegaly and increase VPW suggestive of hydrostatic pulmonary oedema. Patient was discharged home uneventfully, followed up bilirubin was 3.8mg/dL 3 weeks later.

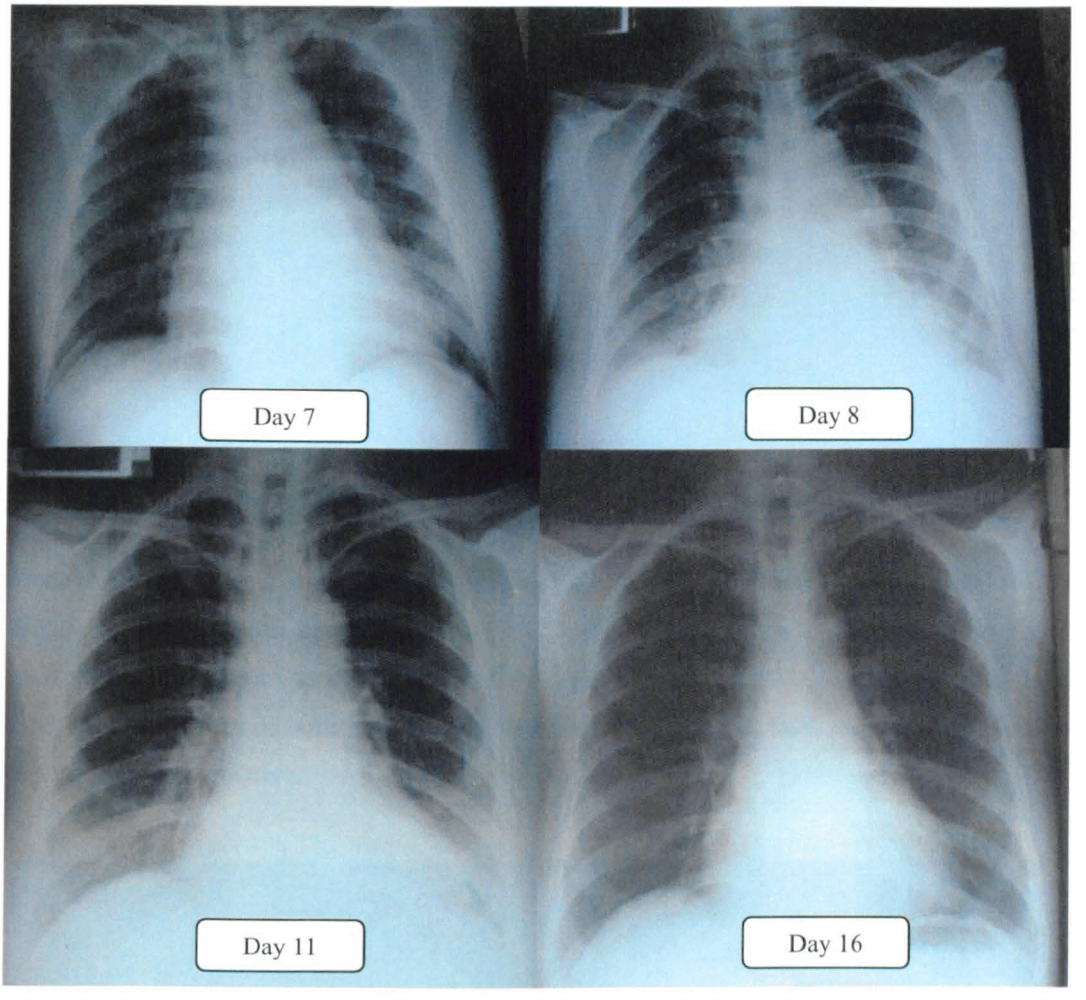


Patient L551: 22 year old man with fever for 2 days, massive haemoptysis developed just before admission, tachypnoea and hypotension on admission. Chest radiograph performed on admission, bilateral diffused nodular infiltrations with cardiomegaly and increase VPW suggestive of hydrostatic pulmonary oedema. Patient developed respiratory failure and cardiac arrest and died quickly hours after admission. *L. interrogans* serovars Autumnalis was isolated from his blood taken on admission.



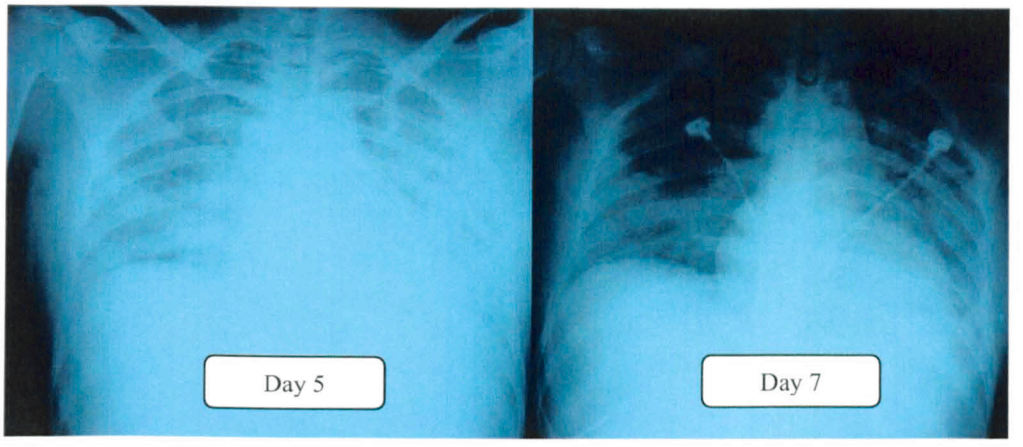
Patient 518: A 59 year old female rice farmer with fever for 4 days. Clinically well on admission, no jaundice, no renal failure, platelet 73×10^9/mL. Developed haemoptysis and respiratory failure 2 days after admission and died one day later. Chest radiograph performed on 2 days after admission, bilateral diffused nodular infiltrations with confluent consolidation, with no cardiomegaly and normal VPW. *L. interrogans* serovars Autumnalis was isolated from her blood taken on admission.

Appendix E: Chest radiographs of patients with leptospirosis and pulmonary oedema

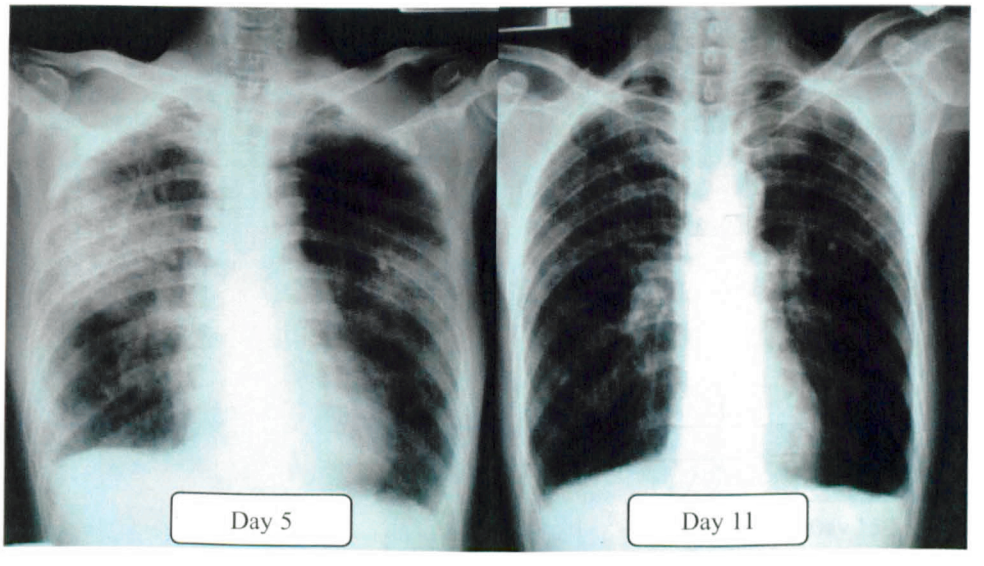


Patient L110: 52 year old female with fever for 6 days, jaundice for 3 days. Dyspnoea and tachypnoea on admission. Creatinine 4.4mg/dL, total bilirubin 14.0mg/dL, platelet 48×10^9/mL.

Appendix F: Chest radiographs of patients with scrub typhus and pulmonary haemorrhage

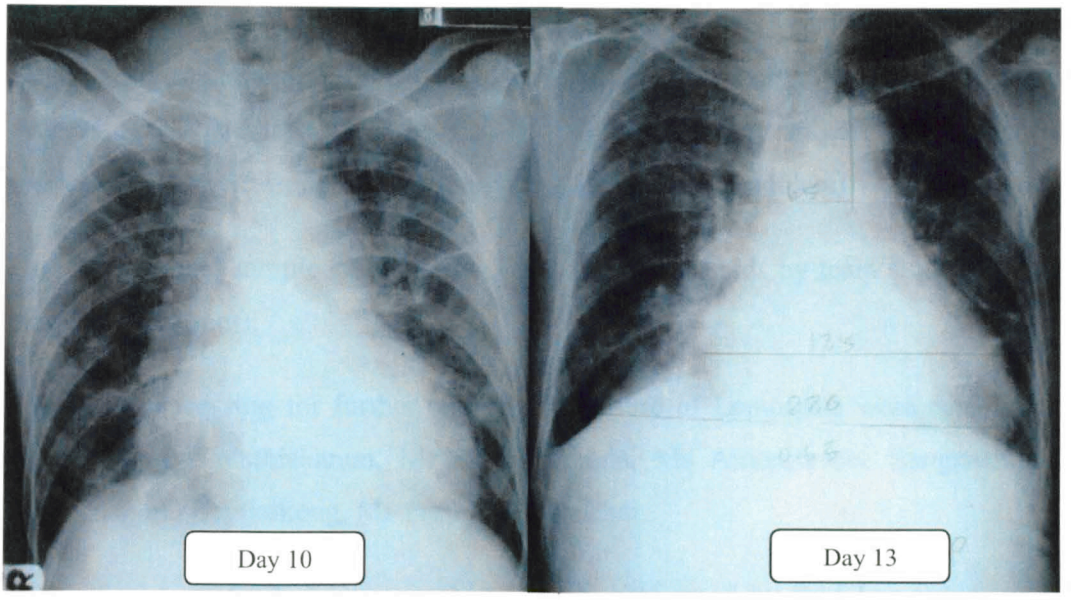


Patient L118: 28 year old near-term pregnant woman presented with fever, dyspnoea, sore throat and blood-streak sputum for 3 days. Supportive treatment in ICU without invasive mechanical ventilation was successful. Patient was discharged home uneventfully after 9 days. Four-fold rising titre of both IgM and IgG together with positive PCR for 16S RNA for scrub typhus were confirmative.

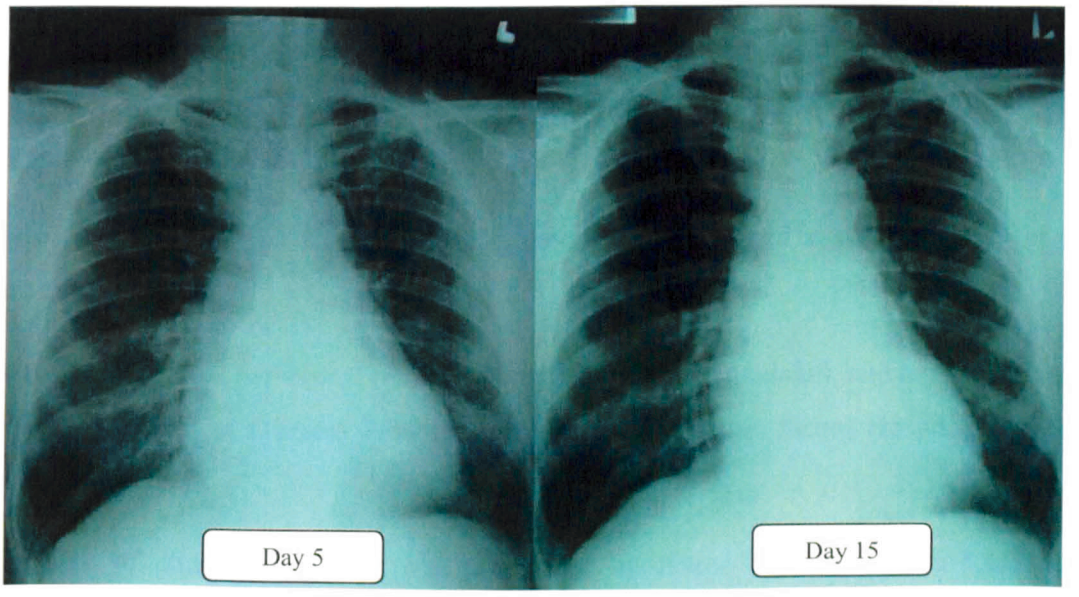


Patient L628: 65 year old man presented with fever, dyspnoea, and blood-streak sputum for 5 days. Hypotension and hypoxia on admission. Supportive treatment in ICU without invasive mechanical ventilation was successful. Patient was discharged home uneventfully after 9 days. Four-fold rising titre of both IgM and IgG for scrub typhus.

Appendix G: Chest radiographs of patients with scrub typhus and pulmonary oedema



Patient L217: 60 year old man came with fever and productive cough for 10 days and alteration of consciousness for 2 days. Serology and PCR for scrub typhus were positive. Patient were recovered and discharged home after 12 days of hospitalisation.



Patient L53: 56 year old man came with fever, headache and productive cough for 5 days. Aseptic meningitis was presented. Serology and PCR for scrub typhus were positive. Patient were recovered and discharged home after 11 days of hospitalisation.

Contributions

Study design, study plan, majority of data and sample collection, part of specimen processing, all database construction, majority of data entry and all data analysis and modeling were performed by Dr Wirongrong Chierakul (Candidate).

Parts of data and sample collection and data entry were aids by team study nurse (Ms. Naowanit Ponpinit).

Specimen processing for further testing and culture of Leptospira were performed by Ms. Vanaporn Wuthiekanun, Mr Sayan Langla, Ms Amornwadee Sangkakam, Ms Thaksinaporn Thaojaikong, Ms Premjit Amornchai.

Serological tests for leptospirosis (MAT) were performed by Prof Lee Smyth and his colleagues at WHO/FAO/OIE Collaborating Centre for the Reference & Research on Leptospirosis, Centre for Public Health Sciences, Queensland Health Scientific Services, Brisbane, Australia, Ms Wimol Petkanchanapong at the National Institute of Health, Ministry of Public Health, Thailand.

Serological tests for scrub typhus and murine typhus (IFA) were performed by Prof Yupin Suputtamongkol and her colleagues at Department of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Biochemical tests and complete blood counts were performed at the laboratory in the hospital.

Coagulogram was performed by Assoc Prof Panatsaya Tientadakul, and her colleague at the Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University.

Review of chest radiographs was performed by Assoc Prof Nitipatana Chierakul, pulmonologist, Department of Medicine, and Assoc Prof Orasa Chawalpralit, radiologist, Department of Radiology, Faculty of Medicine Siriraj Hospital, Mahidol University